Remarks

Claims 1, 5, and 7-8 are currently pending in this application. New claims 7-8 are added in this paper. Support for these new claims can be found, for example, on page 1, lines 6-11 and page 69 (Example 57) of the specification. No new matter has been added.

Applicants respectfully submit that all of the pending claims are allowable for at least the following reasons.

A. Applicants Statement of the Substance of the Interview

A personal interview was held on October 9, 2007. The attendees were Examiners Brian Kwon and Charlesworth Rae, and Mr. Peter Schafer, Ms. Donna Robertson-Chow, and Mr. Hoon Choi, representatives for Applicants. Applicants thank Examiners Kwon and Rae for the courtesy they extended to Applicants' representatives during the interview.

First, the rejection under obviousness-type double patenting was discussed. Applicants' representatives, while stating that they do not agree with the substance of the rejection, offered to file a terminal disclaimer to expedite the prosecution of this application. Examiners agreed that filing a terminal disclaimer would overcome the rejection.

Next, the outstanding enablement rejection was discussed. Applicants' representatives first pointed out that PDE IV inhibition properties of representative compounds were provided in Applicants' previous responses (*see*, *e.g.*, Applicants' response dated January 19, 2006). Based on this, Applicants' representatives pointed out that the "reduction of PDE IV levels," as recited by claim 1 is enabled, a proposition to which the Examiners agreed[1].

With regard to the treatment of an inflammatory and autoimmune disease as recited by claim 5, Applicants' representatives pointed out that post-filing references are available to evidence that the modulation of PDE IV, as shown by the PDE IV data discussed above, is implicated with the treatment of various autoimmune or inflammatory diseases. Examiners agreed that submitting such references would be sufficient to overcome the enablement rejection raised in connection with claim 5 as well.

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[1] Applicants' representatives expressed their concern regarding the allegation made in the Office Action in connection with the enablement rejection. It is alleged in the Office Action that the claims are not enabled even in the face of the PDE IV data provided in Applicants' previous responses, allegedly because a reference purportedly discloses that "simple extrapolation of therapeutic efficacy from animal models of human disorders is not easily feasible." (Office Action, page 15). In this regard, Applicants' representatives respectfully pointed out that such an allegation, which essentially requires human clinical data, is flatly contrary to the well-settled legal principles concerning enablement. Examiners agreed that at least claim 1 is enabled based on the previously submitted PDE IV data.

Finally, Applicants' representatives indicated that Applicants planned to submit the new claims 7-8, which recite a specific compound encompassed by the genus recited by claims 1 and 5. Examiners indicated that such amendments would not present any issues. Each of these points are discussed in more detail below.

B. The Obviousness-Type Double Patenting Rejection Should Be Withdrawn

On pages 8-11 of the Office Action, claims 1 and 5 are rejected under judicially created obviousness-type double patenting over claim 14 of U.S. Patent No. 6,667,316 ("the '316 patent"). Although Applicants respectfully disagree, especially for the reasons set forth in their response of April 24, 2007[2], a terminal disclaimer is submitted herewith solely to expedite the prosecution of this application. In view of the terminal disclaimer, Applicants respectfully request that the rejection of the claims under the obviousness-type double patenting should be withdrawn.

C. The Rejection Under 35 U.S.C. § 112 Should be Withdrawn

On pages 11-18 of the Office Action, claims 1 and 5 are rejected as allegedly failing to comply with the enablement requirement under 35 U.S.C. § 112, first paragraph. The Examiner appears to acknowledge the well-settled legal principle that the "specification disclosure ... must be taken as being in compliance with the enablement requirement unless there is a reason to doubt objective truth of the statement contained therein," yet suggests that there indeed is reason to doubt the truth of the statements contained in the specification. (Office Action, page 15). For the alleged "reason to doubt," the Examiner points to portions of Bielekova *et al.*, *The Journal of Immunology*, 164: 1117-24 (2000) ("Bielekova")[3]. Applicants respectfully traverse this rejection.

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[2] Incorporated herein by reference in its entirety.

[3] Initially provided by Applicants as evidence to support the enablement.

First, Applicants again respectfully invite the Examiner's attention to the PDE IV data repeatedly provided in their previous responses. As discussed during the interview, Applicants respectfully point out that those data, along with the detailed explanation in the specification regarding how the PDE IV inhibition would provide useful therapeutic avenue for the recited diseases, would have provided sufficient enablement for the pending claims. (*See*, *e.g.*, Applicants' response of June 28, 2006, pages 4-10).

In addition, for further evidence, Applicants submit herewith the following references, each of which shows that PDE IV is implicated in the treatment of various autoimmune or inflammatory diseases[4]: 1) Burnouf *et al.*, *Curr. Pharm. Des.*, 8(14): 1255-96 (2002), which indicates that the PDE IV activity is associated with a wide variety of diseases related to inflammatory state or autoimmune pathology (*see*, *e.g.*, Abstract); 2) Dastidar *et al.*, *Curr. Opin. Investig. Drugs*, 8(5): 364-72 (2007), which indicates that PDE IV inhibitors' therapeutic potential in the treatment of various inflammatory disease such as asthma, COPD, psoriasis, rheumatoid arthritis, and inflammatory bowel diseases, e.g., Crohn's disease and ulcerative colitis (*see*, *e.g.*, Abstract); and 3) Sommer *et al.*, *J. Neuroimmunol.*, 79(1): 54-61 (1997), which indicates that PDE IV inhibition is advantageous for the treatment of autoimmune diseases such as multiple sclerosis (*see*, *e.g.*, Abstract). Therefore, as these additional references clearly demonstrate the therapeutic potential of PDE IV inhibition in a wide variety of autoimmune or inflammatory diseases, Applicants respectfully submit that the pending claims are adequately enabled[5].

Finally, for the record, Applicants respectfully offer the following in connection with the legal principles regarding enablement. Applicants respectfully reiterate that the outstanding enablement rejection is legally improper in view of *In re Brana*, 51 F.3d 1560 (Fed. Cir. 1995). (*See*, *e.g.*, Applicants' response of July 20, 2007, pages 4-5, incorporated herein by reference). In response, the Examiner offers the following assertions: 1) *Brana* is distinguishable from the current application in that "no issue of 'asserted utility' [is] present in the instant case and there is no requirement that is being made of applicant to prove the ultimate value in humans" (Office Action, page 5); and 2) facts in *Brana* are distinguishable from those in the current application in that "no in vitro model is well recognized in the art for reliably and predictably determining the anti-PDE IV activity of the instant[ly] claimed compounds against" the recited diseases (*Id.*, page 6). Applicants respectfully disagree with each of those assertions.

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[4] These references are provided in addition to Verghese and Bielekova, both of which are already in the record to show that PDE IV is implicated in the treatment of various autoimmune or inflammatory diseases. (*See* Applicants' response of June 28, 2006, page 7).

[5] The Examiners agreed during the interview that such a showing would overcome the enablement rejection.

With regard to the Examiner's first assertion[6], Applicants respectfully point out that the Examiner has indeed set forth a "requirement that is being made of applicant to prove the ultimate value in humans," contrary to the Examiner's assertion. For example, it appears that while the Examiner acknowledges the well-settled legal principle that the "specification disclosure ... must be taken as being in compliance with the enablement requirement unless there is a reason to doubt objective truth of the statement contained therein," the claims are rejected based on the allegation that there indeed is a "reason to doubt." (Office Action, page 15). The "reason to doubt" provided by the Examiner appears to be a statement from Bielekova that "simple extrapolation of therapeutic efficacy from animal models to human disorders is not easily feasible." (Office Action, page 15) (emphasis in original). Therefore, by premising the enablement rejection on this statement and alleging that it provides a "reason to doubt" that the claims are enabled, the Examiner essentially indicates that human efficacy data are required to establish enablement. Such a requirement set forth by the examiner was precisely at issue in *Brana*, and the current application is not distinguishable from *Brana* in connection with this issue.

With regard to the Examiner's second assertion, *i.e.*, that there is no well-recognized in vitro model regarding the claimed diseases in connection with PDE IV inhibition, Applicants respectfully point out that no evidence is provided by the Examiner to support this assertion. Therefore, to the extent that this statement is made without any objective evidence, Applicants respectfully point out that such an unsupported statement by the Examiner cannot form a basis to reject the claims as allegedly not enabled.

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[6] One point raised by the Examiner in connection with his first assertion is that the current application is distinguishable from *Brana* because no issue of "asserted utility" is present in the current application. However, "asserted utility" is at issue in the current application as well. This is because the Examiner alleges that Applicants' claimed utility of treating autoimmune or inflammatory disease is not enabled. In fact, it is unclear to Applicants how there could be an enablement rejection where there is no issue of "asserted utility."

In sum, Applicants respectfully submit that all of the pending claims are enabled, particularly in view of the PDE IV data and other evidence provided in their previous responses, and additional evidence provided herein. In addition, Applicants respectfully point out that the outstanding enablement rejection is legally improper because the Examiner has not provided any evidence or reasoning acceptable under the current legal principles to rebut the presumption of enablement.

For at least the foregoing reasons, Applicants respectfully submit that the pending claims are enabled and comply with all of the requirements of 35 U.S.C. § 112, first paragraph. Thus, reconsideration and withdrawal of this ground of rejection is respectfully requested.

D. Conclusion

Applicants respectfully submit that all of the pending claims are allowable, and thus, respectfully request that all of the rejections be withdrawn.

No fee is believed due for the submission of this paper. However, if any fees are required for the submission of this paper, or to avoid abandonment of this application, please charge any required fees to Jones Day Deposit Account No. 503013.

Respectfully Submitted,

Date: October 31, 2007

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Therapeutic potential of phosphodiesterase type 4 inhibition in chronic autoimmune demyelinating disease

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Abstract

It was recently demonstrated that selective phosphodiesterase type 4 (PDE4) inhibition suppresses the clinical manifestations of acute experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), and inhibits the production of tumor necrosis factor-α (TNF-α), a pathogenetically central cytokine. Since the most common presentation of MS in humans is a relapsing–remitting course, we investigated the therapeutic potential of PDE4 inhibition in the relapsing–remitting EAE model of the SJL mouse. Administration of rolipram, the prototypic PDE4 inhibitor, reduced the clinical signs of EAE during both the initial episode of disease and subsequent relapses. In parallel, there was marked reduction of demyelination and also less inflammation throughout the central nervous system (CNS) of rolipram-treated animals. Gene expression of proinflammatory cytokines in the CNS was reduced in most of the rolipram-treated animals. Additional experiments demonstrated that PDE4 inhibition acted principally by inhibiting the secretion of Th1 cytokines, however, the encephalitogenic potential of myelin basic protein-specific T cells was not impaired. Our findings suggest that PDE4 inhibitors are a promising cytokine-directed therapy in chronic demyelinating disease. © 1997 Elsevier Science B.V.

Keywords: Cytokine; Experimental autoimmune encephalomyelitis; Multiple sclerosis; Phosphodiesterase type 4; Rolipram; Tumor necrosis factor

1. Introduction

In experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS), a number of highly specific therapies have been developed, based on the central role of the trimolecular complex of autoreactive T cell, autoantigenic peptides and the appropriate MHC-product (Martin et al., 1992a; Wekerle et al., 1994). Interference with each partner of this complex, such as the blocking of antigen receptors on autoreactive T cells with specific antibodies, leads to a marked suppression of disease activity in inbred rodent models (Acha-Orbea et al., 1989). However, the T cell response in a genetically heterogeneous human population shows a much greater variety and complexity (Martin et al., 1992b; Giegerich et al., 1992; Utz et al., 1994). At present, the applicability of such immunotherapies to human disease remains uncertain.

Promising new treatment approaches for T cell-mediated autoimmune diseases are directed against proinflammatory cytokines. This is based on growing experimental evidence that tumor necrosis factor-α (TNF-α), the closely related lymphotoxin-α (LT), and interferon-γ (IFN-γ) are involved in the development of inflammation and tissue damage (Selmaj and Raine, 1988; Vassalli, 1992; Renno et al., 1995). The treatment of EAE and other autoimmune models with anti-TNF/LT antibodies or soluble TNF-receptors has been shown to be remarkably effective (Ruddle et al., 1990; Selmaj et al., 1995). Furthermore, we and others showed recently that substances such as rolipram, a selective cyclic nucleotide phosphodiesterase type 4 (PDE4) inhibitor, pentoxifylline, a nonspecific PDE inhibitor, or phosphatidylserine, a natural membrane phospholipid, are able to suppress TNF-production in vitro and

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EAE in vivo (Sommer et al., 1995; Genain et al., 1995; Rott et al., 1993; Monastra et al., 1993). Targeting PDE4 seems particularly promising, because this isozyme is predominantly, though not exclusively, present in inflammatory cells. The potential clinical impact of this approach could be enormous, because it targets a central pathogenic process that by-passes complex antigen receptor-specific immunoregulatory mechanisms.

Our prior study in the Lewis rat left a number of questions unanswered (Sommer et al., 1995). First, in that model, EAE is characterized by a single episode of disease, unlike the relapsing and remitting course seen in the SJL mouse EAE and in human MS; therefore the effects of rolipram on chronic disease could not be assessed. Second, the SJL mouse model of EAE has much more demyelination upon histologic examination compared with EAE in the Lewis rat, which again is more reminiscent of MS (Martin et al., 1992a). In this study, we show that long-term PDE4 suppression ameliorates clinical signs and pathological changes in chronic relapsing EAE in SJL mice, although autoreactive T cells are not eliminated from the rolipram-treated mice.

2. Materials and methods

2.1. Reagents

Rolipram: racemate and enantiomers were kindly provided by Dr. Helmut Wachtel, Schering AG, Berlin. For in vitro studies, rolipram was dissolved in a 0.25% solution of dimethylsulfoxide:saline (1:10) and further diluted in culture medium. For treatment studies, rolipram racemate was dissolved in saline and injected intraperitoneally (i.p.).

Myelin basic protein (MBP) was prepared from guinea pig spinal cords (Rockland, Gilbertsville, PA) as previously described (Deibler et al., 1972).

2.2. Mice

Female SJL/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were 8–12 weeks old when used for experiments. All procedures were in compliance with guidelines set by the NIH Animal Care and Use Committee.

2.3. Induction of EAE

EAE was induced according to previously published protocols (Racke et al., 1991). Briefly, mice were immunized with 400 μg MBP in complete Freund's adjuvant. Ten days later, MBP-specific lymph node cells (LNC) were cultured at 4×10^6 cells per ml in RPMI-1640 containing 10% FCS, nonessential amino acids, HEPES, 2-mercaptoethanol, glutamine, antibiotics, plus 25 μg/ml MBP for 4 d. Cells were then washed and injected at 3×10^7 in 0.2 ml PBS per mouse i.v. For serial adoptive transfer experiments, spleen cell suspensions were cultured and transferred in the same way.

2.4. Treatment schedules and clinical rating

Mice were treated with 1×6.25 mg/kg (experiment 1) or 3×6.25 mg/kg (experiment 2) per day rolipram (racemate) i.p. in 0.2 ml saline from day 2 after cell transfer until the end of the observation period. Control animals were injected with saline alone. Clinical signs of EAE were ranked on a scale from 0 (healthy) to 5 (tetraparetic) (Racke et al., 1994). A relapse was noted when the clinical score worsened by at least one point for at least two consecutive days.

2.5. Gene expression

After perfusion with saline, brain and spinal cord were removed and RNA extracted from whole CNS tissue using RNAzol (Cinna/Biotecs, Friendswood, TX). RNA was purified and the coupled reverse transcriptase (RT)–PCR performed as described (Svetic et al., 1991; Racke et al., 1994). Briefly, RNA samples were reverse transcribed with Superscript RT (BRL, Rockville, MD) and cytokine-specific primers were used to amplify selected cytokines. For each gene product, the optimum number of cycles was determined experimentally. To verify that equal amounts of RNA were added in each RT-PCR reaction, primers for hypoxanthine phosphoribosyl transferase (HPRT) were used in each experiment. Amplified PCR products were detected by Southern blot analysis.

2.6. Neuropathology

Animals were perfused with phosphate-buffered 2.5% glutaraldehyde as described (Racke et al., 1991). Briefly, CNS tissue was removed and thin slices were made of all levels of the neuroaxis. These were post-fixed in cold 1% osmium tetroxide for 1 h, dehydrated in ethyl alcohol and embedded in epoxy resin (Epon 812). One micrometer epoxy sections of tissue were stained with toluidine blue and examined by light microscopy in a blinded fashion.

2.7. Cell culture

Proliferative responses from LNC and spleen cells were performed by incubating 8×10^5 cells/well in triplicate or quadruplicate with or without MBP (12.5–100 μg/ml, maximum proliferation usually at 25 μg/ml) or Con A (2 μg/ml) in 96 well flat-bottom microtiter plates (Costar, Cambridge, MA) at 37°C with 5% CO_2. Cells were pulsed with 1 μCi methyl-^{3}H[thymidine] (Amersham, Arlington Heights, IL) per well for the last 16 h of the culture period and harvested on fiber filters (after a total incubation time of 96 h, if not stated otherwise). Incorporated radioactivity was measured on a Betaplate counter (Wallac, Gaithers-

Table 1
Summary of clinical data [a]

	First attack (d 7–21)		Relapse (d 22 and later)	
	incidence of EAE	MMS (±sd)	incidence of relapse	MMS (±sd)
Experiment 1 (treatment from day 2):				
Control	16/16 (100%)	3.69 (±0.58)	13/13 (100%)	3.46 (±1.01)
Rolipram (1 × 6.25 mg/kg)	12/20 (60%)	2.05 (±1.63) [b]	15/17 (88%)	2.59 (±1.09) [c]
Experiment 2 (treatment from day 2):				
Control	11/13 (85%)	2.62 (±1.27)	6/9 (67%)	1.67 (±0.94)
Rolipram (3 × 6.25 mg/kg)	3/13 (23%)	0.46 (±0.93) [b]	1/9 (11%)	0.44 (±0.96) [c]
Experiment 3 (treatment from day 17):				
Control	6/6 (100%)	3.08 (±0.45)	6/6 (100%)	2.75 (±0.62)
Rolipram (1 × 6.25 mg/kg)	6/6 (100%)	3.08 (±0.34)	4/6 (67%)	2.00 (±0.41) [b]

[a] Three representative treatment experiments are shown. Note that treatment was started on day 2 after induction in experiments 1 and 2, but only on day 17 (after peak of the first attack) in experiment 3. Animals were treated until day 58 (experiment 1), 38 (experiment 2), or 62 (experiment 3), respectively. Abbreviation: MMS mean maximum score.

[b] $p < 0.05$.

[c] $p < 0.001$.

burg, MD). For cytokine determination 8×10^6 lymph node cells were cultured in 24 well plates at 2 ml per well with or without 25 μg/ml MBP. After 12, 24, 48 or 96 h, cell free supernatants were frozen at −70°C until use. Determination of TNF-α and IFN-γ was performed by commercial ELISA (Genzyme, Cambridge, MA). Proliferation of the CTLL-2 cell line was used to detect IL-2 (Bottomly et al., 1991). IL-4 was determined using the IL-4 dependent CT.4S cell line (Seder et al., 1992) and was found to be below detection limits throughout. TGF-β was measured by a CCL-64 bioassay (Danielpour et al., 1989).

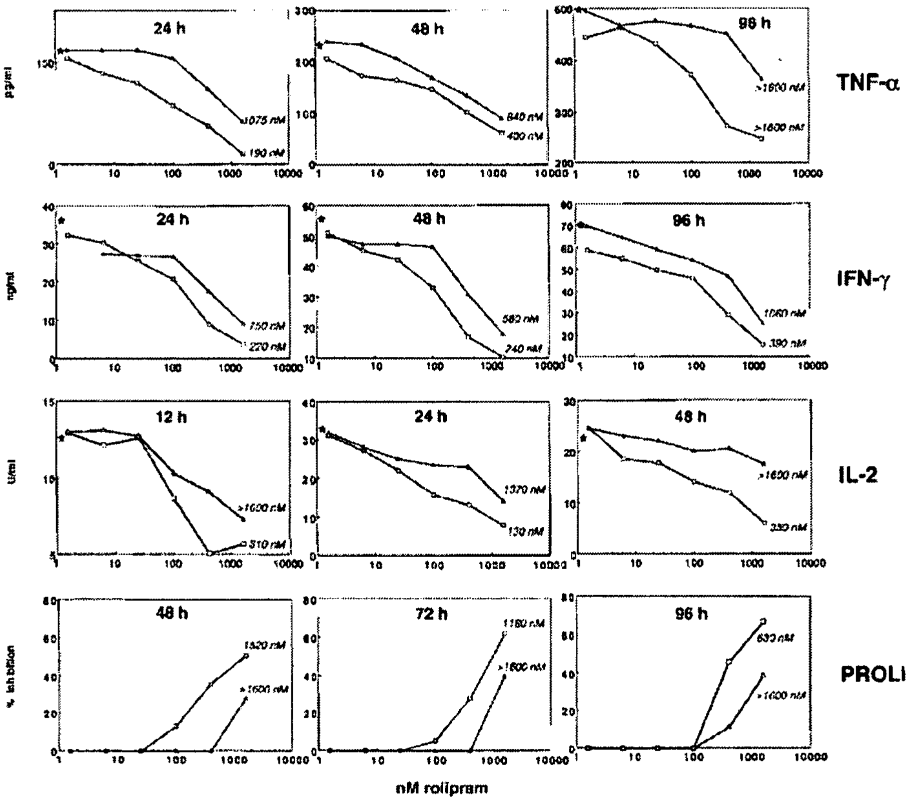

Fig. 1. Rolipram stereospecifically suppresses production of Th1 cytokines by encephalitogenic lymph node cells in tissue culture supernatants. MBP-specific LNC were cultured with MBP (25 μg/ml) and the given concentrations of rolipram: open squares = (−)-rolipram, open triangles = (+)-rolipram. Asterisks indicate the cytokine production without rolipram. Numbers in italics give the respective IC_{50} value.

3. Results

3.1. *Effect of rolipram on MBP-primed lymph node cells in vitro*

Using two enantiomers of rolipram, the production of the Th1 cytokines, IL-2, IFN-γ, and TNF-α was suppressed in a stereoselective manner, with (−)-rolipram being more potent (Fig. 1). In addition, IL-4 and TGF-β were also examined in the cell culture supernatant of the MBP-specific LNC, but were only detected in negligible amounts and unchanged by rolipram (not shown). A much higher concentration of rolipram was required to inhibit T cell proliferation than Th1 cytokines. Fifty percent inhibition of T cell proliferation by the more potent (−)-enantiomer required 600–1600 nM, whereas for Th1 cytokines this was achieved by 100–400 nM (Fig. 1). Viability of LNC, tested by trypan blue exclusion, was not impaired at concentrations of 400 nM or less or (−)-rolipram.

3.2. *Effect of in vivo administration of rolipram on chronic EAE*

Administration of rolipram following transfer of MBP-primed lymphocytes suppressed the clinical signs of chronic-relapsing EAE (Fig. 2, Table 1). Injections beginning two days after the adoptive transfer of encephalitogenic T cells resulted in lower incidence and significantly reduced severity of clinical disease in a dose-dependent manner. This effect was apparent during the first episode of disease and also during subsequent relapses.

However, it was important to observe whether rolipram would have a treatment effect, if administered after the signs of EAE were already established. In experiment 3 (Fig. 2c, Table 1) 12 mice were randomized into two groups after the initial attack and treated from day 17 post transfer. With this regimen the mean maximum score of the rolipram treated group was also reduced significantly.

3.3. *Histopathologic evaluation of rolipram treated mice*

To examine the effects of rolipram on histopathology in EAE, particularly with regard to its effect on demyelination, representative mice from experiment 2 (Table 1) were sacrificed on day 15 post transfer. Mice that received vehicle treatment displayed perivascular infiltrates associated with demyelination (Fig. 3a–b, Table 2). Some remyelination was also evident in the late acute phase of EAE. There was a marked reduction of demyelination and a modest reduction in cellular infiltration in the CNS of the rolipram-treated animals (Fig. 3c–d, Table 2). Secondary Wallerian degeneration was mainly found in untreated animals, but was very mild in the rolipram-treated group. Results from the rolipram- and the vehicle-treated groups are summarized in Table 2. Representative sections are shown in Fig. 3. The decrease in inflammation in the rolipram-treated mice was similar to our prior study in the Lewis rat (Sommer et al., 1995).

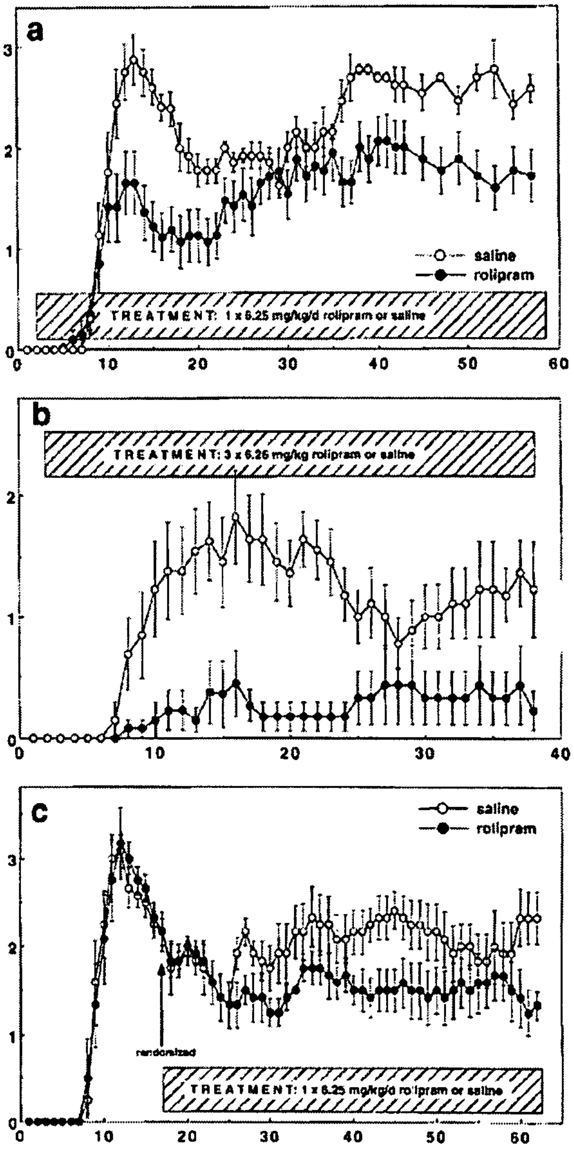

Fig. 2. Rolipram suppresses chronic-relapsing EAE. Mean clinical scores (±sem) are shown for treatment experiments 1 (a), 2 (b), and 3 (c). The hatched bars give the duration and dose of treatment. Note that in experiment 3 treatment was started during recovery from the first attack after randomization of the animals into two groups with similar clinical course. Mean maximum scores and statistical evaluation of the three experiments are shown in Table 1.

3.4. *Rolipram treatment does not deplete MBP-specific T cells*

Spleen cells from various animals in the treatment experiments were incubated in vitro for 4 d with 25 μg/ml MBP and then transferred to naive mice. Table 3 shows, that all animals, including those from the rolipram

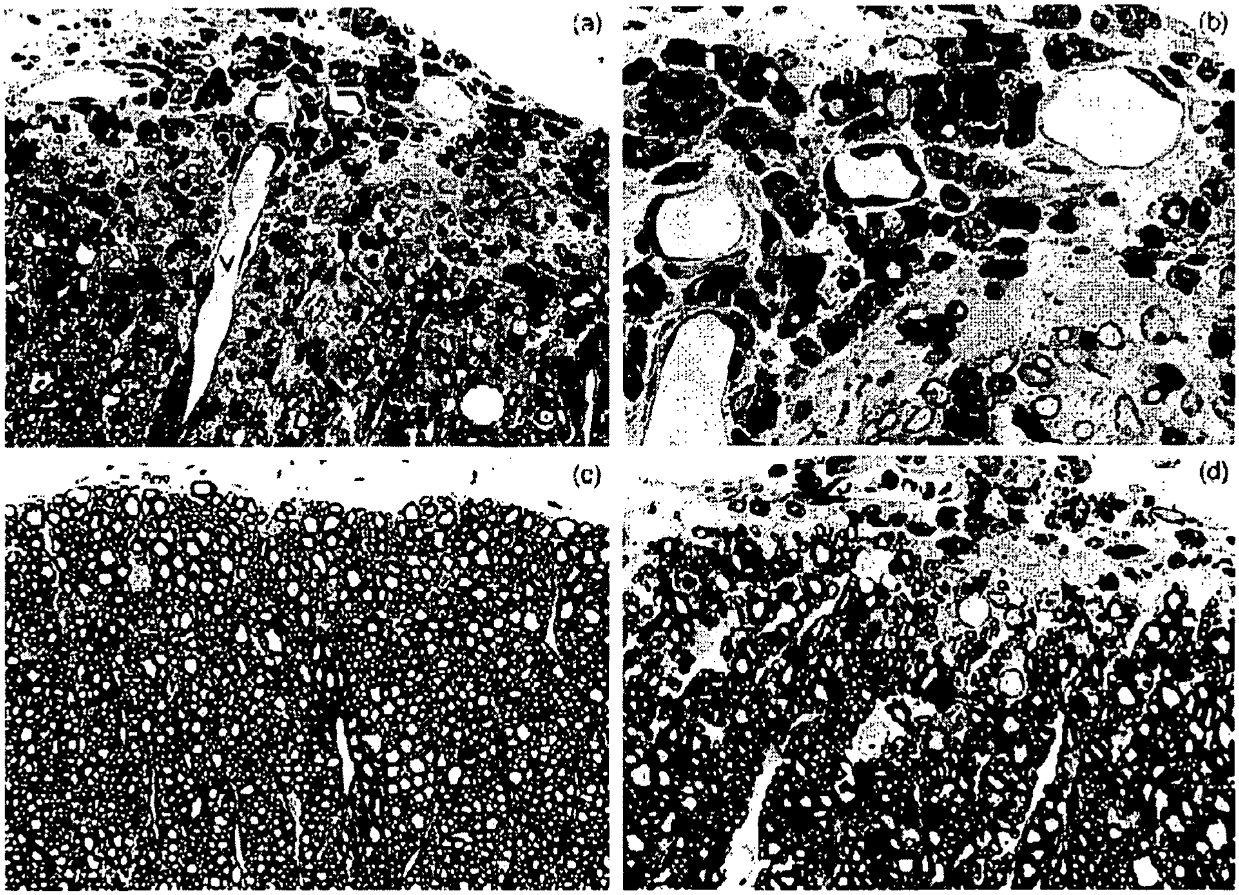

Fig. 3. Pathology in EAE with and without rolipram treatment. Representative animals from treatment experiment 2 treated with 3 daily doses of rolipram or saline (see Fig. 2, Table 1) were investigated on day 15 post transfer of MBP-specific LNC. A. Placebo treated EAE; 15 days post transfer (dpt); clinical grade 2. An area of demyelination flanks a penetrating subpial vessel (v) in an anterior column of the lumbar spinal cord. Note the perivascular and subarachnoid infiltration and the many demyelinated axons (arrows); ×350. B. Detail from A. Note the many myelin debris—laden macrophages in the leptomeningeal space and the demyelinated axons (arrows) in the underlying lesion. Some fibers display thin myelin sheaths which are probably indicative of remyelination at this late acute phase of the disease (clinical onset, 6 dpt); ×875. C. Rolipram-treated EAE; 15 dpt; clinical grade 0. In this treated animal that showed no signs of clinical EAE, the CNS parenchyma displays no changes and there is no inflammation; ×350. D. Rolipram-treated EAE; 15 dpt; clinical grade 1. A different animal from C with minimal signs of disease. Note the moderate degree of inflammation in the subarachnoid space, the small demyelinated lesions (curved arrows), the scattered myelin ovoid indicative of Wallerian degeneration (small straight arrows), and the hypertrophic astrocytes (large clear cells); ×350.

Table 2
Histopathologic analysis [a]

Treatment group	Vehicle		Rolipram	
	inflammation [b]	demyelination [c]	inflammation	demyelination
CNS location				
cerebrum/cerebellum	+ + WD	+	+ – + +	±
cervical/thoracic cord	+ – + + WD	+ – + +	± – +	±
lumbar/sacral cord	+ – + + WD	+ + re	±	±

[a] Rolipram treatment reduces demyelination and inflammation compared with control mice. The table summarizes CNS pathology from representative mice (two per group) in experiment 2 (treated three times daily) obtained on day 15 days after cell transfer (see Table 1, Fig. 3 for further details).
[b] Inflammation was scored according to the following system: –, no inflammatory cells; ±, a few inflammatory cells; +, organization of inflammatory infiltrates around blood vessels; + +, increasing severity of extensive perivascular cuffing with extension into the adjacent subarachnoid space.
[c] The extent of demyelination was determined according to the following scoring system: –, no demyelination; ±, a few, scattered, naked axons; +, small groups of naked axons; + +, large groups of demyelinated axons. Abbreviations: WD, Wallerian degeneration; re, remyelination.

Table 3
Summary of serial transfer experiments [a]

Donor mice			Recipient mice		
mouse	treatment	score	incidence of EAE	day of onset	max. score
day 14:					
1	rolipram	0	1/1	7	4
2	rolipram	0	2/2	7, 9	2, 3
3	vehicle	2	2/2	8, 8	3, 4
4	vehicle	2	1/3	11	2
day 28:					
1	rolipram	0	1/1	5	4
2	rolipram	4	1/1	11	5
3	vehicle	4	1/1	12	3

[a] Spleen cells from rolipram treated mice can transfer EAE as effectively as those from control mice. Spleen cell single cell suspensions from animals of various treatment groups (day 14-donor mice had been treated three times daily, day 28-donor mice once daily) were incubated with MBP (25 μg/ml) for four days and then transferred (3×10^7 cells) to naive mice to test their encephalitogenic potential. Depending on the number of spleen cells obtained from an individual donor mouse, from one to three mice could be the recipient of a serial transfer of splenocytes. The table indicates the number of mice that developed EAE from each individual donor mouse (1/3 = 3 mice received activated splenocytes, 1 developed EAE).

treatment group without any neurological deficit, could transfer EAE as effectively as control MBP-specific splenocytes. These data demonstrate that rolipram does not lead to deletion of encephalitogenic MBP-specific T cells, even after 4 weeks of continuous treatment (Table 3).

3.5. *Cytokine gene expression in the CNS*

Gene expression of cytokines associated with encephalitogenicity and immune regulation was examined in the CNS of rolipram-treated mice from both treatment experiments by RT-PCR. Despite considerable heterogeneity in cytokine expression in individual mice IL-2, TNF-α (except for one outlier) and IFN-γ, was decreased in most of the rolipram-treated animals (Fig. 4). IL-4 was only detected at very low levels in the animals investigated.

4. Discussion

In this paper, we extend our previous findings by showing that continuous PDE4 inhibition reduces clinical signs in a chronic–relapsing model of EAE. The clinical course and the histopathologic changes observed in the SJL mouse model of EAE used here resemble those in multiple sclerosis. Furthermore, we demonstrate that reduction of demyelination is a hallmark of PDE4 inhibition (Fig. 3, Table 2). In addition, serial transfer experiments and CNS cytokine determination in this study provide further evidence for the hypothesis that Th1 cytokine suppression by rolipram is probably important in mediating its clinical effect. Experiments in the Lewis rat model have shown that the in vivo effect of PDE4 inhibition on EAE is clearly stereospecific (N. Sommer, unpublished).

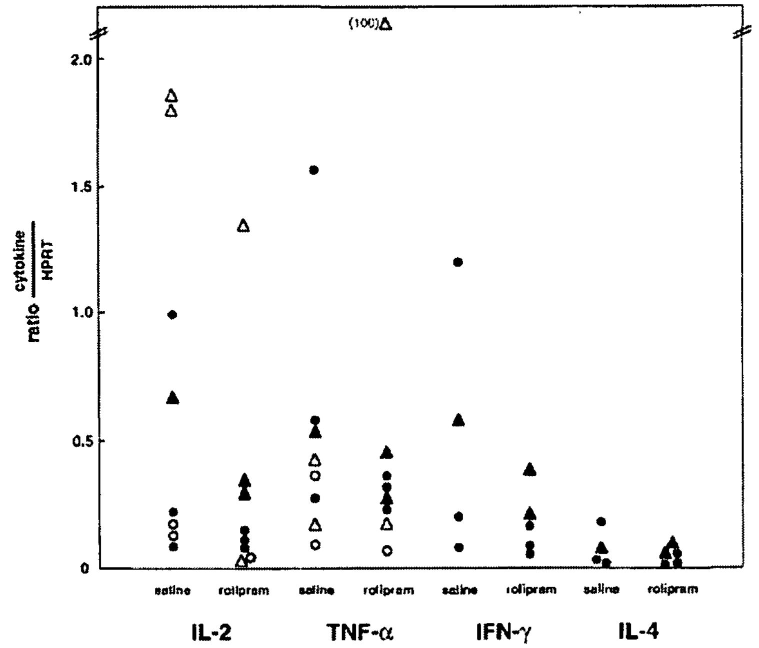

Fig. 4. Cytokine gene expression in the CNS. Cytokine gene expression was evaluated by performing Southern blots on RT-PCR cDNA from RNA isolated from the CNS of selected mice from treatment experiments 1 or 2 (treatment once daily = triangles, three times daily = circles) either on days 14–16 (closed symbols) or days 24–28 (open symbols). Values are expressed as ratio of cytokine signal divided by the constitutively expressed HPRT. In naive control mice no cytokine signal could be detected.

Based on the now well established role of TNF-α in the pathogenesis of EAE and MS, a cytokine-directed treatment approach seems reasonable and applicable to human disease (Genain et al., 1995; Sommer et al., 1995). Indeed, TNF-neutralizing treatment approaches have been followed with great effort and have reached clinical trials. For instance, in rheumatoid arthritis, anti-TNF antibody treatment using a chimerized human/mouse monoclonal antibody has already shown promising results (Maini et al., 1995). A note of caution must be made to the clinical use of macromolecules, such as monoclonal antibodies or soluble cytokine receptors. Despite their great potential, they have some typical disadvantages, namely unfavorable pharmacokinetics, parenteral administration, and high production costs. Also, long-term administration of monoclonal antibodies may not always neutralize the biologic effects of TNF. At low concentration, they may stabilize the biologically active trimeric structure of TNF and prolong its effects (Maini et al., 1995).

Therefore, novel approaches to identify low molecular weight inhibitors of cytokine production have been pursued intensively. Cyclic AMP has been known to be involved in T cell activation for some years (Wang et al., 1978; Marcoz et al., 1993). Furthermore, increasing intracellular cAMP by adenylate cyclase stimulation, phosphodiesterase inhibition, prostanoids or dibutyryl cAMP are known to have anti-inflammatory activity, including TNF-inhibition (Semmler et al., 1993; Sinha et al., 1995). Cyclic nucleotide phosphodiesterase (PDE) inhibitors are particularly interesting candidates as immunomodulating drugs, because an increasing number of even more selective and potent substances is currently being developed (Beavo et al., 1994). Among the seven PDE families the PDE4 isozymes have received the greatest attention in this respect. Inhibition of PDE4 isozymes by rolipram was their original defining property, and compared with other PDEs, they present the most complex picture concerning biochemical properties and tissue distribution. Thus far, four PDE4 genes have been identified (PDE4A/B/C/D) (Beavo et al., 1994). Rolipram is among the most potent PDE4 inhibitors described to date and has been extensively studied as long term therapy in patients with depression (Wachtel and Schneider, 1986). It has minor toxicity in humans, particularly no general immunosuppression was observed; however, some dose-limiting effects consisted of gastrointestinal symptoms, such as nausea and emesis (Zeller et al., 1984). Undoubtedly, the ongoing development of new selective PDE4 subtype inhibitors could increase tissue selectivity and clinical potency, while reducing side-effects. So far, both message and protein for PDE4A and PDE4B, but not PDE4C or PDE4D, have been shown to be up-regulated in human monocytes by activation of protein kinase (Torphy et al., 1995).

The precise molecular mechanism of rolipram action is not yet clear. Current evidence suggests that it inhibits the production of proinflammatory cytokines, best shown for TNF-α (Prabhakar et al., 1994; Genain et al., 1995; Sommer et al., 1995; and this study). Here, we have shown that gene expression of several proinflammatory cytokines is reduced in the target organ, the CNS (Fig. 4). These data must be interpreted with caution, since they depend on differences in the magnitude and cell types of the inflammatory infiltrates and do not necessarily reflect protein levels of the respective cytokines. Our data, in accord with data from a monkey model of EAE (Genain et al., 1995) and in vitro findings in human monocytes (Prabhakar et al., 1994), suggest that rolipram may act, in part, by inhibiting TNF-α and IL-2 production in vivo. Furthermore, it is now generally agreed that cell types involved in inflammatory processes contain PDE4 in relevant quantities (Torphy et al., 1992), though some inflammatory cells may also contain PDE3. Preliminary data suggest that in human MBP-specific Th1 cells PDE4 is more prevalent than PDE3 and correlates well with cellular activation processes (Ekholm et al., unpublished). This study provides further support for the concept that PDE4 inhibition may be useful as a therapy for diseases mediated by Th1 cytokines, such as multiple sclerosis.

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Recent Advances in PDE4 Inhibitors as Immunoregulators and Anti-Inflammatory Drugs

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Abstract: The phosphodiesterases (PDEs) are responsible for the hydrolysis of intracellular cyclic adenosine and guanosine monophosphate (cAMP and cGMP, respectively). They are classified into 11 major families (PDE1-11) and the type 4 phosphodiesterase (PDE4) is a cAMP-specific enzyme localized in airway smooth muscle cells as well as in immune and inflammatory cells. The PDE4 activity is associated with a wide variety of diseases some of which have been related to an inflammatory state, (e.g. asthma, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis (RA)) while others have recently been connected to autoimmune pathology. Therefore, an intense effort toward the development of PDE4 inhibitors has been generated for the last decade. Unfortunately, the effects of prototype PDE4 inhibitors have been compromised by side effects such as nausea and emesis and the clinical use of those compounds is still limited. Several companies have focused on the design of a new generation of PDE4 inhibitors dissociating beneficial activity and adverse effects. This review highlights the recent data of the most advanced clinical candidates, the design and structure activity relationships of the recent structural series reported in the literature over the last two years, as well as recent advances in the multiple therapeutic indications of PDE4 inhibitors (a review with 375 references).

INTRODUCTION

The cyclic nucleotides cAMP and cGMP are intracellular second messengers that mediate many physiological processes. At high concentration these nucleotides bind to specific protein kinases (PKA and PKB). The subsequent activation of these kinases induces the phosphorylation of a wide variety of substrates that modify cellular responses. The levels of the cyclic nucleotides are regulated by the rates of the related synthesis and degradation: the synthesis is generated from ATP and GTP by adenylate and guanylate cyclases that are activated by G-protein coupled receptors and the degradation is linked to phosphodiesterase activity. The PDEs comprise a large superfamily of proteins whose members are responsible for the hydrolysis of cAMP and cGMP to the corresponding inactive nucleoside 5'-monophosphates AMP and GMP, respectively. The classification of PDE enzymes into 11 families with several different species including subtypes and splice variants has been described [1-3]. Very recently, much progress in the PDE field has been provided with the discovery and the characterization of several new phosphodiesterase genes [4] including PDE7 [5-7], PDE8 [8-9], PDE9 [10], PDE10 [11-15] and PDE11 [16-17]. The availability of phosphodiesterase isoenzymes as pure recombinant proteins has greatly facilitated the identification of potent, selective inhibitors. The potential of these inhibitors to therapeutically exploit the molecular diversity of the phosphodiesterases has progressed significantly [18].

cAMP specific PDE4 has emerged as the predominant isoenzyme in inflammatory cells and in pulmonary smooth muscle cells. The PDE4 family is comprised of four genes (PDE4A to D) [19] with a unique chromosomal location per gene [20-21]. Due to alternative splicing of the genes, multiple splice variants are reported and classified into two main groups, the long and short forms. All subtypes provide a conserved catalytic domain (~270 amino acids) and the absence or presence, respectively for short and long form, of two additional regions UCR1 (upstream conserved region 1) and UCR2 within the N-terminus [22-23]. The four subtypes are found differentially expressed among tissues and cells [24-25]. PDE4A, PDE4B and PDE4D gene products are found in most immune and inflammatory cells [25-32]. PDE4A, PDE4D and PDE4C gene are expressed in airway epithelial cells [33-34]. The four genes are expressed in the brain showing a differential distribution within the regions [31, 35-36].

During the last decade, numerous preclinical studies have demonstrated the modulation of inflammatory and immunocompetent cell activation by selective PDE4 inhibitors, supporting their potential use as emerging therapies in various inflammatory and immune diseases. More recently, a few clinical trials have confirmed the hypothesis that PDE4 inhibitors may provide benefit in the treatment of chronic airway diseases such as asthma and COPD. This review highlights the most recent advances in the design of PDE4 inhibitors as a new class of anti-inflammatory and immunomodulatory drugs.

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EFFECTS ON INFLAMMATORY CELLS

The recruitment of activated inflammatory cells at the site of injury is one of the key characteristics of inflammation. While this process remains essential for the homeostasis, in some particular conditions, cell recruitment becomes excessive and contributes to the worsening and the chronicity of the disease. Activation of inflammatory cells is characterized by the release of various pro-inflammatory mediators such as histamine, leukotrienes, granule proteins, oxygen-derived metabolites, cytokines which exert a deleterious effect on effector tissues. PDE4 is the most represented PDE in inflammatory cells and the inhibition of PDE4 activity has been extensively studied in these cell types generating a wide range of *in vitro* and *in vivo* data.

Basophils

PDE3, PDE4 and PDE5 are present in human basophils [37]. Selective inhibition of PDE4 in this cell type leads to the down-regulation of anti-immunoglobulin E (IgE)-induced histamine and leukotriene C_4 (LTC_4) release [38-39] with a maximum of activity around 50 to 60% inhibition. An inhibitory effect on interleukin (IL)-13 and IL-4 release from mite allergen-stimulated asthmatic human basophils was also described with rolipram [40].

Mast Cells

Few data only are available regarding the effects of non selective PDE inhibitors in primary human mast cells, where PDE3, PDE4 and PDE5 are all expressed. For example, 3-isobutyl-1-methylxanthine (IBMX) produces a strong inhibition of anti IgE-induced cysteinyl leukotriene and prostaglandin D_2 (PGD_2) release from mast cells, an effect that is reported not to be due to PDE4 inhibition [39]. In mast cell like cultured human cord blood mononuclear cells, selective PDE4 inhibitors inhibit granulocyte macrophage-colony stimulating factor (GM-CSF), IL-5 and macrophage inflammatory protein 1α (MIP1α) release but not histamine or PGD_2 release following anti-IgE stimulation [41-42]. No effects are reported regarding proteases, platelet-activating factor (PAF), adenosine, tumor necrosis factor α (TNFα) and other mast cell derived cytokines.

Eosinophils

Both PDE3 and PDE4 are represented in human eosinophils. Particularly, PDE4A, B and D subtypes but not PDE4C are expressed in this cell type [29]. Eosinophils, that play an important role in the pathophysiology of asthma, have been extensively studied in the context of PDE4 inhibition. Selective PDE4 inhibitors block superoxide and reactive oxygen species formation [43-47] and LTC_4 release [48-49]. The production of granule proteins such as eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) is down-regulated by PDE4 inhibitors alone [50-51] while the inhibition of eosinophil peroxidase (EPO) release requires a combination of the PDE4 inhibitor with salbutamol [52]. Complement factor C5a-, PAF- and eotaxin-induced eosinophil chemotaxis as well as PAF-, eotaxin- and GM-CSF-induced CD11b (α_M subunit of Mac 1) up-regulation can also be inhibited by PDE4 inhibitors [51, 53-54]. Finally, the effect of PDE4 inhibitors on IL-5-induced eosinophil survival was also demonstrated [51]. *In vivo*, the effect of PDE4 inhibitors on eosinophil recruitment was even more studied using different models. In sensitized and ovalbumin challenge mice, PDE4 inhibitors significantly block eosinophil but also lymphocyte and neutrophil recruitments, prevent IL-4 and IL-5 accumulation in the bronchoalveolar lavage (BAL) fluids and changes in airway functions [55]. PDE4 inhibitors also suppress sephadex- [56], respiratory syncitial virus (RSV)-induced [57], LTD_4- [58], IL-5 [59] and antigen-induced eosinophil infiltration into the lungs of guinea pigs [45, 58, 60-62], rats [63] ferrets [64] and monkeys [65-66].

Neutrophils

Neutrophils play an important role in inflammatory diseases, accumulating rapidly at the site of injury or infection. In neutrophils, cAMP is primarily hydrolyzed by PDE4 and PDE4B2 is the predominent vatiant [67]. PDE4 inhibitors suppress a range of neutrophil functions, including superoxide production [38, 68], granule secretion [38], IL-8 release [69], adhesion molecule expression [54], chemotaxis and adhesion [68, 70], human lung elastase release and expression [71] and LTB_4 biosynthesis [49, 72] but do not compromise phagocytosis or microbial killing [68]. Inhibition of PDE4 with appropriate activation of adenylate cyclase is sufficient to inhibit induction of E-selectin expression on human lung microvascular endothelial cells to a level that has functional consequences for neutrophil adhesion [73]. *In vivo*, PDE4 inhibitors block lipopolysaccharide (LPS)-induced neutrophilia in bronchoalveolar lavages of rats [74-75] and guinea pigs [76-77], ovalbumin-induced BAL neutrophilia in sensitized mice [55] and rats [78] and LPS-induced [^{111}In]-polymorphonuclear cell trapping in the lungs of rabbits [79].

Monocytes/Macrophages

The importance of mononuclear phagocytes in inflammation is due to their complementary and interrelated functions, blood monocytes being the circulating precursors of tissue macrophages. While PDE4 is predominent in human monocytes, PDE3 activity increases and PDE1 and 5 appear when monocytes differenciate into macrophages [80-82]. One of the major effects of PDE4 inhibitors is probably their ability to down-regulate LPS-induced TNFα production. While this inhibitory effect on TNFα is complete and extremely potent in monocytes [38, 83] it becomes less pronounced in macrophages [47, 80] probably due the decline of PDE4 activity in this cell type. One study reports an increase in total cAMP PDE activity in monocytes taken from mild asymptomatic asthmatics compared to healthy subjects, reflected by an increase in the proportion of PDE3 and a decrease in the proportion of PDE4. However, this augmented enzyme activity was not associated with an alteration in the ability of PDE4 inhibitors to prevent TNFα release from monocytes in asthmatics compared to healthy individuals [84]. PDE4 activity is upregulated in activated human peripheral blood mononuclear cells (PBMCs) [85]; furthermore, in human monocytes activated by LPS, it was suggested that only the PDE4B gene was induced and that this upregulation could be inhibited by IL-10 [67, 86]. PDE4 inhibitors block in a lesser extent the release of IL-1β [87-88] or GM-CSF [89] from primary monocytes or human PBMCs stimulated with LPS. Contradictory data are reported on IL-10 release with either an enhancing effect of PDE4 inhibitors when PBMCs are stimulated with LPS [90] or an inhibitory effect when PBMCs are stimulated with phytohemagglutinine (PHA) [91]. The production of LTB_4, LTC_4 and the release of arachidonate from human monocytes are also affected by PDE4 inhibitors [92-93]. A few studies on human macrophages report little or no effects of selective PDE4 inhibitors on respiratory burst, thromboxane B_2 (TXB_2) or LTB_4 release [94-96] while in guinea pigs alveolar macrophages, significant inhibitory effects were shown on arachidonate release [97]. *In vivo*, numerous studies report the ability of PDE4 inhibitors to reduce TNFα release in the blood or in BAL fluids of various species [63, 75-76, 98].

Endothelial Cells

Endothelial cells play a major role during inflammation by allowing inflammatory and immunocompetent cell to migrate from the circulation to the inflamed tissues. This process includes the increased permeability of endothelial layers and the up-regulation of adhesion molecules on the surface of endothelial cells. Inhibition of PDE4 with appropriate activation of adenylate cyclase is sufficient to inhibit induction of E-selectin expression on human lung microvascular endothelial cells (HLMVEC) to a level that has functional consequences for neutrophil adhesion. In contrast, combined inhibition of PDE3 and 4 isoenzymes is necessary to block vascular cell adhesion molecule (VCAM)-1 and to have inhibitory effects on eosinophil adhesion to activated HLMVEC. Finally, upregulation of intercellular adhesion molecule (ICAM)-1 expression on HLMVEC does not appear to be modulated by PDE3 and PDE4 inhibition [73]. Similarly in human vascular endothelial cells in culture, rolipram in combination with forskolin inhibits TNFα-, LPS- or phorbol myristate aceter (PMA)-induced E-selectin expression but has little effect on ICAM-1 expression [99]. The effects of PDE4 inhibitors on plasma extravasation were mainly studied in *in vivo* models of pulmonary inflammation in rats, mice and guinea-pigs. PDE4 inhibitors suppress leakage induced by several stimuli, including antigen, PAF, histamine, LPS and IL-2 [100-105].

Epithelial Cells

Epithelial cells actively participate in inflammatory disease by liberating mediators such as arachidonate metabolite and cytokines. Human bronchial epithelial cells express a low total PDE activity, made up predominantly of PDE1 and PDE4 [106]. PDE5 as well as PDE7 are also detected in primary cultures of human airway epithelium. In these cells, enhanced cAMP levels, induced by forskolin and PDE4 inhibition, increased the formation of PGE_2, but not of IL-8 or 15-hydroxyeicosatetraenoic acid (15-HETE) whereas increased cGMP levels provoked by PDE5 inhibition reduced the PGE_2 synthesis [34]. A selective PDE4 inhibitor can significantly block TNFα and GM-CSF but not IL-8 release from bronchial epithelial cells isolated from COPD patients [107]. *In vivo*, PDE4 inhibitors have been reported to exert a protective action on epithelium and to modulate tissue remodeling following ozone or antigen exposure, probably by down-regulating the activity of various proteins such as matrix metalloproteinase (MMP)-9 and IL-6 [76, 108-109].

EFFECTS ON IMMUNE CELLS

Dendritic Cells

Only few studies report the effects of selective PDE4 inhibitors on dendritic cells. During differenciation of human monocytes to dendritic cells, a drastic decrease in PDE4 activity was observed, while activities of PDE1 and PDE3 substantially increased. In these cells, selective PDE4 inhibitors block the production of TNFα in response to LPS stimulation. Moreover, rolipram, in combination with a selective PDE3 inhibitor produced a synergistic inhibitory effect on CD4+ T cell proliferation in response to antigen presentation by dendritic cells [47, 110].

Lymphocytes

T lymphocytes play a key role in coordinating the immune response. These cells express PDE4, PDE1, PDE3, PDE5 and PDE7. The distribution of PDE4 activity appears to be similar between CD4+ and CD8+ lymphocytes [29] but elevated levels of PDE4 mRNA as well as increased cAMP PDE activity have been reported in the CD4+ T cells of atopic donors compared to non atopic [111-112]. Another study reports the presence of PDE4A, PDE4B and PDE4D isoforms in CD4+ and CD8+ lymphocytes with no significant differences observed between healthy and asthmatic subjects [113]. While the expression of the various PDE4 isoforms has been identified in T helper (Th)-1 and Th-2 clones [114], it is suggested that Th-2 antigen-specific T-cell clones are more sensitive to PDE4 inhibition than Th-1 antigen-specific clones [115] and that PDE4A and PDE4B play a major role against T cell proliferation [83]. In general, PDE4 inhibitors prevent anti CD3/anti CD28-, PHA-, PMA- or antigen-induced T cell proliferation, IL-2, IL-4, IL-5, IL-13 interferon (INF)-γ production [38, 47, 116-121], PAF and IL-8 induced T cell chemotaxis [122]. IL-10 production from human PBMCs was shown to be reduced by PDE4 inhibitors, probably indirectly as a result of their inhibitory effect on TNFα release [91].

The effects of PDE4 inhibitors on B lymphocytes have been less studied. B cells contain majoritary PDE4 and PDE3 [29, 123]. Recent studies report inhibitory effects of rolipram on IL-4-induced IgE synthesis from human PBMCs, but no effects when rolipram is incubated with purified B cells only [124] suggesting a mediation via monocytes or T cells. Interestingly, B cell proliferation following stimulation with LPS and IL-4 was potentiated by rolipram [125]. Finally, no effects have been reported so far on other immunoglobulins.

THERAPEUTIC POTENTIAL IN INFLAMMATORY AND IMMUNE DISEASES

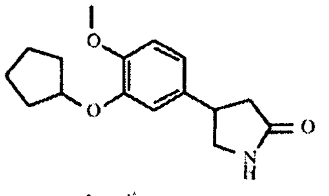
Because of the dual capacity of cAMP to down-regulate inflammatory and immune cell activation and to mediate smooth muscle cell relaxation, PDE4 inhibitors have first been designed for the treatment of chronic inflammation of the airways such as asthma and COPD [126-130]. While PDE4 inhibitors are relatively poor bronchodilators compared to β_2-adrenoceptor agonists, their potential as agents susceptible to treat the inflammation underlying chronic airway diseases has gained the interest of numerous pharmaceutical companies and has been the subject of several recent reviews [131-139]. More recently, their potential as immunomodulatory agents has gained further interest [140-141] and several hypotheses have been built regarding their role in allergic disorders [142], autoimmune diseases [143] and other inflammatory diseases [144-158].

The therapeutic potential of PDE4 inhibitors has been extensively investigated in preclinical models of rheumatoid arthritis (RA) [159], atopic dermatitis [160], multiple sclerosis [161-165], Crohn's disease [166], colitis [167], acute lung injury [168], inflammatory pain [169-170], lymphoid malignancies [171-173], hypertension [174], osteopenia [175], erectile dysfunction [176], anxiety [177], diabetes [178] and even infections with HIV [179-180].

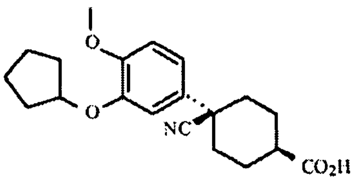
DRUG DESIGN STRATEGIES

Clinically, the effects of PDE4 inhibitors including the prototypical rolipram (**1**) have been limited by side effects such as nausea and vomiting that occurred at therapeutic plasma levels [181-183]. Other effects have been reported, mainly such as increased gastric secretion and CNS-driven side effects [182, 184-188]. To date, no selective PDE4 inhibitor is on the market. Rolipram, early discovered as a potent and selective PDE4 inhibitor, was the main starting template used for the synthesis of new derivatives [189]. A wide variety of analogs were synthesized but unfortunately providing more or less similar side effect profile than their precursor rolipram. In the last decade, companies with an interest in the PDE4 area have focused on the design of a new generation of candidates providing an improved therapeutic window dissociating beneficial anti-inflammatory activity and emetic side effects. For this purpose, various strategies still are under investigation.

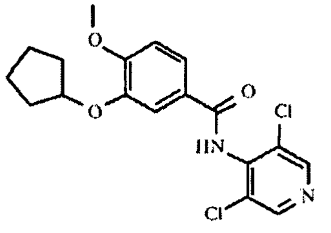
One of the first strategies is the identification of inhibitors structurally unrelated to rolipram [190]. Another strategy focuses on the discovery of candidates selectively inhibiting one of the two conformers of PDE4. This approach is based on the discovery that PDE4 may exist in two different conformations. Historically, it was reported that in addition to binding at the catalytic site of PDE4 (Ki ~1 μM), rolipram was found to bind an additional site with high affinity (Ki ~1 nM) named HARBS (High Affinity Rolipram Binding Site) [189]. Further scientific debate concluded that the two binding sites represent two conformers of PDE4 that were termed LPDE4 (Low affinity rolipram binding PDE4) or LAR (Low Affinity for Rolipram) and HPDE4 (High affinity rolipram binding PDE4) or HAR (High Affinity for Rolipram) [131-132, 137, 191-194]. Using a novel fluorescence resonance energy transfer (FRET)-based equilibrium PDE4 binding assay, recent data demonstrated that the two conformations result from the reversible PDE4 binding to a cofactor (Mg^{2+}). In this study, cAMP and PDE4 inhibitors including (R)-rolipram, cilomilast (**2**), piclamilast (**3**) and CDP-840 (**4**) displayed a differential binding affinity to the apoenzyme (free enzyme) and to the holoenzyme (enzyme bound to Mg^{2+}). The Mg^{2+} binding induces a PDE4 conformational change that produces a high affinity interaction with cAMP and therefore activates the catalytic mechanism [195]. It was suggested that HPDE4 conformation predominates in CNS



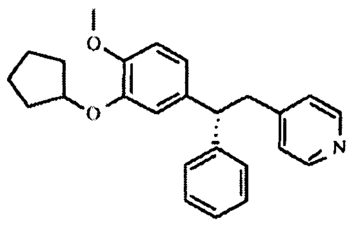
1 : rolipram



2 : cilomilast



3 : piclamilast



4 : CDP-840

[196], whereas LDPE4 is much more important than HPDE4 in immune and inflammatory cells [197-199]. Several studies (but not all) suggested that PDE4 inhibitors associated to LPDE4 conformation are related to beneficial activity [131, 191, 200-205] while candidates being connected to the HPDE4 conformation provide various PDE4 adverse effects [194, 206-210].

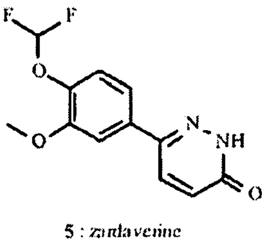
The presence of multiple subtypes and splice variants, coupled with differential expression and regulation among tissues and cells, provides a wide range of opportunities for the development of a new generation of selective inhibitors that should dissociate between beneficial activity and adverse effects. Therefore, a related strategy attempts to identify inhibitors targeting one PDE4 subtype that could lead to increase tissue and cell selectivity, to decrease side effects and finally to improve the therapeutic index [23-24, 211]. However, there are challenges associated with this approach: firstly, the high sequence homology between the subtypes and secondly the choice of the subtype to be targeting [23]. Cilomilast (**2**) is a unique compound in clinical phase trials providing a weak PDE4D selectivity, 10 fold versus other PDE4 subtypes [212].

A different route for reducing PDE4 side effects concerns an inhaled administration of inhibitors in order to decrease systemic exposure [213]. However, the mixed PDE3/PDE4 inhibitor zardaverine (**5**) is associated with some PDE4 specific events of nausea and vomiting [214]. Zardaverine clinical development was discontinued and the approach using inhaled PDE4 inhibitors is not without its challenges.

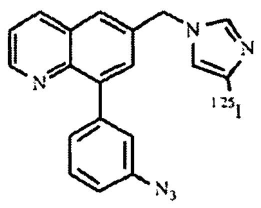
Very recently, data showed that the disease activated drug (DAD) concept could lead to a novel system for treating chronic inflammatory diseases with a reduced side effect profile. DAD drugs are a derivatization of one active principle or a combination of two covalently linked drugs. DAD drugs in contrast to prodrugs release their active principle(s) only when they reach the inflamed tissues. Thus, this approach is supposed to show many advantages including the diminution of side effects. The DAD concept was evaluated in asthma using an isoquinoline derivative as an inhaled PDE4 inhibitor. This inhibitor was either derivatized into prodrugs or combined with corticosteroids [215]. In fact, reduced systemic side effects of inhaled DADs were observed and might be associated to the local release of the active moiety in inflamed lung. Moreover, using DAD as a combination between a PDE4 inhibitor and a steroid in clinic, a reduced clinical effective dose was observed and reported as a result of a synergy of potencies connected to the liberation of the two anti-inflammatory actors.

Studies from Merck Frost suggested that, at least in ferret, PDE4 inhibitors through the cAMP elevation in sympathetic neurons induce a similar emetic response to alpha$_2$-adrenoceptor antagonists. The authors suggested that PDE4 inhibitors increasing intracellular levels of cAMP may modulate the level of mediators such as noradrenaline, 5-HT and substance P and may be responsible for inducing side effects [216-218]. Therefore, emesis produced by PDE4 inhibitors may be evaluated through the appropriate emetic response in ferrets or through the anesthetic reversal effect in rats. Merck Frost was the first group having identified a photoaffinity probe (**6**) emetic in ferrets, efficacious and competitive with other PDE4 inhibitors [219]. This probe should be useful for the identification of the emesis and efficacy targets of PDE4 inhibitors.

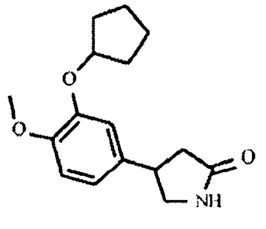
Inflazyme described a method for enhancing the therapeutic activity of PDE4 inhibitors by reducing their emetogenic properties [220]. The method is made up of a benzylation of PDE4 inhibitors: suitable benzylation chemistry is to extract a hydrogen from a PDE4 inhibitor, preferably with a base, and then react the resulting nucleophilic PDE4 inhibitor with a benzylating agent. The benzyl group may be attached to either a carbon or nitrogen



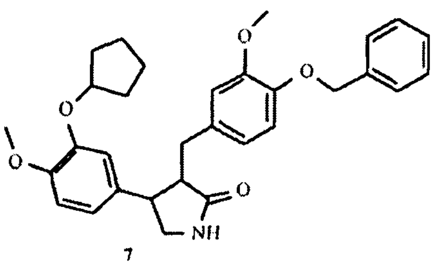
5 : zardaverine



6 : PDE4 radiolabeled inhibitor



1 : rolipram



7

atom of a PDE4 inhibitor. The benzylated rolipram analog (7) was > 10-fold less emetic than rolipram. It was potent as rolipram on PDE4 enzyme with IC_{50} values of 0.40 and 0.42 μM but provided a 60-fold lower affinity for the HARBS with respective IC_{50}s of 0.36 and 0.0065 μM. The synthesis of several reference PDE4 inhibitors was reported.

The 3D-structure of the catalytic domain of PDE4B2B was recently resolved [221]. The catalytic domain folds into a novel compact structure composed of 17 helices and 3 subdomains. The PDE4B2B crystal structure, as a representative of this therapeutically important class of enzymes, provides binding and specificity information that should be very useful in drug design. Very recently, molecular docking models for cAMP and rolipram, using the PDE4B2B crystal data, were reported [222].

Without any crystal structure, the mutation analysis strategy was assessed to identify amino acids involved in the PDE4 binding activity and selectivity. For example, Asp333 was mutated in HSPDE4D3 in connection with mutations that modify the rolipram sensitivity in RNPDE4B1 [223]. The results showed that Asp333 is involved in the inhibitor binding site. Following a sequence comparison between PDE4 and cGMP-specific PDEs showing that PDE4D3 Asp333 corresponds to a conserved asparagine in cGMP enzymes, the PDE4D3 Asp-Asn mutant was prepared, evaluated and found able to hydrolyse cGMP. In addition, the PDE4D3 Asp-Ala mutant showed a propensity to hydrolyze cGMP. All these data concluded that this Asp residue is also involved in the nucleotide discrimination.

Another approach for designing novel PDE4 inhibitors is to construct a peptidic binding site model using a training set of diverse chemical structures [224], (see diazepino-indoles in chapter of "New Chemical Series in PDE4 Field").

RECENT ADVANCES ON CLINICAL DEVELOPMENT OF THE MAIN SELECTIVE PDE4 INHIBITORS

Rolipram (1) is known as the standard of PDE4 inhibitors. Meiji Seika discontinued rolipram as a nootropic agent during development [225]. Rolipram was licenced from Schering for the potential treatment of depression. Evaluated in phase 2 trials for treatment of tardive diskinesia in Japan, it was also discontinued. Rolipram was investigated for its anti-inflammatory effects in asthma, but was discontinued as well for CNS and cardiovascular side effects [226].

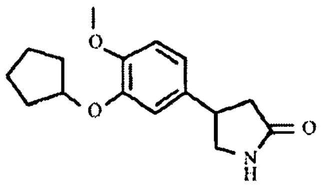
Cilomilast (2, Ariflo®, SB-207499) is developed by GlaxoSmithKline. Cilomilast is in phase 3 clinical trials as a potential treatment for asthma and chronic obstructive pulmonary disease (COPD). A 12-month, double-blind, placebo-controlled, extension study was conducted to evaluate the long-term safety, tolerability and efficacy of cilomilast in patients with asthma [227-228]. Patients received cilomilast 10 mg BID or placebo for an initial eight weeks when the dose could be increased to 15 mg BID; patients then continued for a further ten months. Clinically relevant improvements above placebo were seen in pulmonary function test parameters from week 1 sustained through month 12. Diary asthma symptoms indicated improvements in cough, wheeze and breath-lessness/chest tightness in the cilomilast group compared to placebo. Cilomilast was associated with small increases in the occurrence of headache, abdominal pain, and nausea. Cilomilast is also in Phase 3 development for the treatment of COPD. Cilomilast 15 mg BID demonstrated improvement in lung function and health status and was safe and well-tolerated during a 6-week phase 2 trial. In this study, cilomilast significantly improved lung function as measured by a 10 percent improvement (130 mL) in FEV_1 from baseline and was generally well tolerated with the most common treatment-related adverse events being nausea, diarrhea and abdominal pain [229]. This first demonstration of clinical efficacy in COPD patients was recently confirmed in a 6-month trial in which patients received cilomilast 15 mg p.o. BID or placebo. This study demonstrated that cilomilast significantly improved health status as characterized by a reduction in the St. George's Respiratory Questionnaire (SGRQ) total score of 4.1 points [230], improved lung function (averaged 40 mL improvement of FEV_1 versus placebo over the duration of treatment [231], and reduced the risk of exacerbation by 39% relative to placebo [232]. Cilomilast was safe and well tolerated in this study; the few gastrointestinal adverse events reported by cilomilast-treated patients were generally self-limited and mild or moderate in intensity [233].

The Byk Gulden PDE4 inhibitor, Roflumilast (**8**, BY-217), has completed phase 2 asthma studies and is in phase 3 for asthma and COPD. Data has recently been released to indicate that a single dose (1 mg) of the compound inhibits the late asthmatic reaction in bronchial allergen challenge [234], and that 4 weeks treatment with a single oral daily dose (500 μg) inhibits exercise-induced bronchospasm [235]. The first study consisted in a randomized two period crossover study comparing the effects of a single dose of roflumilast versus placebo after allergen challenge. Roflumilast was administered 1 hour prior to the allergen challenge. Mild asthmatics on β_2-agonist treatment only were challenged with allergen using standard protocols. Data of twelve patients who experienced both an early asthmatic reaction (EAR) and late asthmatic reaction (LAR) were analyzed. The maximal LAR to the respective allergen was significantly ($p < 0.05$) inhibited by 62%. Interestingly, there was also a trend in to inhibit the EAR. The second study, a placebo-controlled, randomized, double-blind, two-period, crossover study, was performed to investigate the safety and efficacy of roflumilast in 16 patients with exercise-induced asthma. The patients received placebo and roflumilast (500 μg/day) in random order for 28 days each. In both study periods, exercise challenge was performed 1 h after dosing on days 1, 14 and 28. FEV_1 was measured before the test and repeatedly up to 12 min after the end of exercise challenge. Blood samples for determination of LPS-stimulated TNFα in whole blood *ex vivo* as a surrogate parameter for the inhibition of inflammatory cell activation were taken predose on days 1 and 28. Serial safety measurements were performed during both study periods. Statistical analyses showed a significant superiority of roflumilast over placebo on day 28. The mean percentage fall of FEV$_1$ after exercise was reduced by 41% as compared to placebo. An improvement of lung function during roflumilast treatment was also observed on days 1 and 14. The median TNFα level decreased by 21% during roflumilast treatment, but remained essentially constant under placebo. Roflumilast was safe and well tolerated during this study. Another study examined the effects of roflumilast on vigilance, visual orientation, motor coordination and concentration. The PDE4 inhibitor was tested at a dose of 500 µg p.o. in the morning for 7 days in a double-blind, randomized, placebo-controlled, crossover study in 18 healthy volunteers. Safety-related performance in traffic and at work was similar on roflumilast and placebo in this trial [236]. A recent publication describes the efficacy of roflumilast in allergic rhinitis [237]. In this randomized, placebo-controlled, double-blinded, crossover study, 25 subjects with histories of allergic rhinitis but asymptomatic at screening received roflumilast (500 µg once daily) and placebo for 9 days each with a washout period of at least 14 days in between treatment periods. In each of the treatment periods, controlled intranasal allergen provocation with pollen extracts was performed daily beginning the third day of treatment, each time approximately 2 hours after study drug administration. Five and 30 minutes after each allergen provocation, rhinal airflow was measured by means of anterior rhinomanometry and the subjective symptoms obstruction, itching, and rhinorrhea were assessed by means of a standardized visual analog scale. Rhinal airflow improved almost consistently during the 9 days of roflumilast treatment, and it was significantly higher at study day 9 on roflumilast in comparison with placebo, a result also found for itching and rhinorrhea. With respect to the subjective obstruction score, a significant difference in comparison with placebo could be demonstrated within 4 days. This study showed that roflumilast effectively controls symptoms of allergic rhinitis and that PDE4 inhibitors might be a future treatment option not only in allergic asthma but also in allergic rhinitis or the combination of the 2 diseases. A 6-month placebo controlled COPD study is currently ongoing but no results have been reported so far.

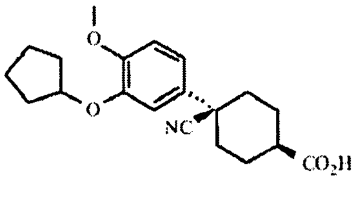
Arofylline (**9**, LAS-31025, Almirall Prodesfarma) was initially reported in clinical trials for treatment of asthma by oral route. Arofylline was discontinued at phase 3 in this therapeutic indication. However, in a double-blind, placebo-controlled phase 3 trial in patients with moderate to severe COPD, inhaled arofylline (90 mg daily for three months) significantly showed improvement of lung function (improved FEV1 and reduced incidence of exacerbations). The most common side effect was reported to be transient gastrointestinal effect [238].

The development of piclamilast (**3**, RP-73401, Novartis, IC$_{50}$ ~1nM)) as an inhaled drug against asthma was discontinued at phase 2 for reason of undesirable side effects and poor pharmacokinetics. Piclamilast was evaluated for the treatment of RA as well. Particularly, RP-73401 displayed a reduction of TNF-α and IL-12 release, an increase of the anti-inflammatory cytokine IL-10, a diminution of T-cell responses and a direct protective effects on bones [239].

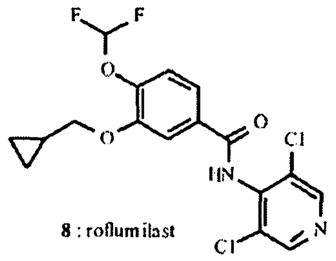
BAY-19-8004 (**10**, Bayer) is a benzofuran derivative recently discontinued in clinical phase 2 for the treatment of asthma [240]. The development for COPD has been suspended until all phase 2 findings are complete. With respect to the different PDE4 preparations, BAY-19-8004 provided PDE4 IC$_{50}$s between 50 and 500 nM [241]. *In vivo*, this compound showed efficacy in various animal models of asthma and COPD. In a LPS-induced lung neutrophilia model in rats, Bay-19-8004 was more efficient than cilomilast (**2**) in inhibiting the increase of neutrophils in the bronchoalveolar lavage (BAL) with an ED$_{50}$ value at 0.6 mg/kg *p.o.* in comparison to 16 mg/kg *p.o.* [242]. In guinea-pigs, the Bayer compound blocked the ovalbumin-induced eosinophil migration (ED$_{50}$ at 1.5 mg/kg *p.o.*) and reduced the immediate ovalbumin-induced bronchoconstriction (61% at 3 mg/kg *p.o.*) [243]. In the repeated treatment model, BAY-19-8004 (0.1 mg/kg *p.o.* once a day for 10 days) significantly prevented the development of the airway hyperresponsiveness and partially inhibited antigen-induced eosinophilia in BAL [244].



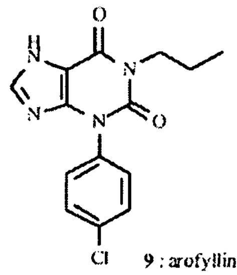
1 : rolipram



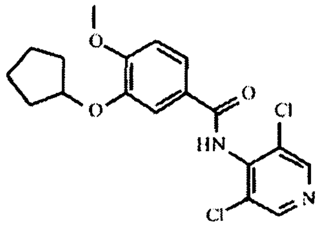
2 : cilomilast



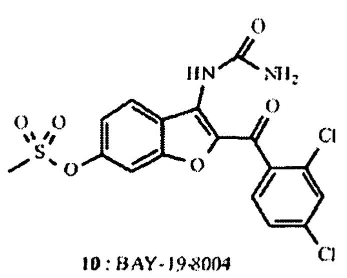
8 : roflumilast



9 : arofylline



3 : piclamilast



10 : BAY-19-8004

Finally, conscious ferrets showed no emetic responses at doses reached 30 to 60 fold the efficacy dose [243].

Cipamfylline (**11**, Leo Pharmaceuticals, BRL-61063 or HEP-688) is a selective PDE4 inhibitor currently in phase 2 for the treatment of atopic dermatitis. Previously, SmithKline Beecham discontinued the development of **11** subsequent to phase 1 clinical trials in which doses > 7 μg/kg induced transient nausea, vomiting and headache.

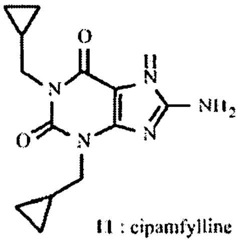
Mesopram (**12**, Schering AG, ZK-117137 or SH-636), a PDE4 inhibitor structurally related to rolipram, is in phase 2 trials for the treatment of relapsing-remitting multiple sclerosis. Recently, the immunomodulatory activity of mesopram was reported in preclinical rodent models. Particularly, this compound selectively inhibited Th1 cell activity with no effects on cytokine production or Th2 cell proliferation [245]. Atizoram (**13**, CP-80633, Pfizer) was originally developed as an oral treatment of asthma, but discontinued in this indication due to emesis in humans at plasma concentration exceeding 0.16 μg/ml [246]. This compound showed efficacy in phase 2 clinical trials for atopic dermatitis [247].

CI-1018 (**14**, Parke Davis, Pfizer), a benzodiazepine compound, showed a good potency and efficacy *in vivo* versus antigen-induced pulmonary eosinophilia model in Brown-Norway rats with an ED$_{50}$ value at 5 mg/kg after oral administration. This compound was not emetic in several species including ferrets: CI-1018 induced no vomiting episodes up to 10 mg/kg *i.p.* whereas rolipram was emetic at 0.1 mg/kg *i.p.* [248-250]. This compound was discontinued at phase 1 for reasons of potential teratogenicity [251]. CI-1044 (**15**, PD-189659, Parke Davis, Pfizer) is a back-up to CI-1018. CI-1044 inhibited TNF-α release from human peripheral blood monocytes and human whole blood *in vitro* with IC$_{50}$ values at 0.34 and 0.84 μM, respectively [252-255]. *In vivo*, this selective PDE4 inhibitor showed higher inhibitory activity than cilomilast in the antigen-induced eosinophil recruitment in Brown-Norway rat with an ED$_{50}$ value at 3.2 mg/kg *p.o.* (cilomilast was inactive at 10 mg/kg *p.o.*). In the LPS-induced TNF-α release model in wistar rats, CI-1044 was equipotent to cilomilast with ED$_{50}$s at 2.8 and 3 mg/kg *p.o.*, respectively. After a 10 mg/kg intravenous administration, CI-1044 induced no emetic side effects in contrast to cilomilast. CI-1044, after oral administration in rats, was active in inhibiting LPS-induced TNF-α release *ex vivo* [256]. These *ex vivo* data suggested that TNF-α should be used as a marker in clinical studies for evaluating the efficacy of PDE4 inhibitors in blocking inflammatory processes. Very recently, CI-1044 was reported to inhibit plasma TNF-α release in LPS-stimulated whole blood of healthy volunteers and COPD patients. The efficacy of CI-1044 in this assay was slightly better than that of rolipram and much higher than that of cilomilast in the same subjects [257]. These data indicated that TNF-α is an appropriate marker of the anti-inflammatory effects of PDE4 inhibitors in COPD patients and that CI-1044, providing significant anti-inflammatory properties in the context of COPD, may prove efficacious in the treatment of lung inflammation.

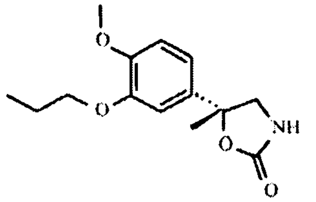
Kyowa Hakko has started phase 1 clinical trials in Europe with the PDE4 inhibitor, KW-4490 (no structure reported), for asthma [258].

CDP-840 (4, Celltech Group / Merck Frost) was discontinued in phase 2 due to unpromising efficacy [146].

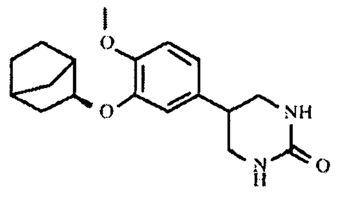
Merck Frosst and Celltech collaborate in the development of a series of PDE4 inhibitors for the potential treatment of asthma, rheumatoid arthritis and COPD [259]. An undisclosed compound was in clinical trials in Europe for the potential treatment of asthma (April 2001). Then, the compound was reported in phase 1 trials (June 2001) and in phase 2 trials (October 2001).



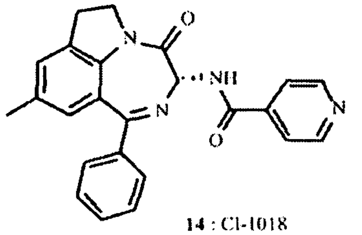
11 : cipamfylline



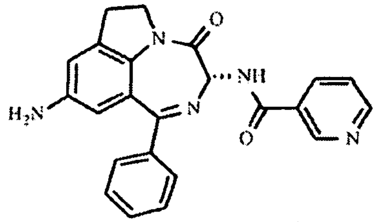
12 : mesopram



13 : atizoram



14 : CI-1018



15 : CI-1044

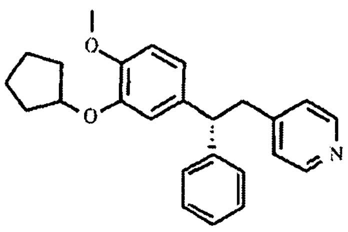
Merck Frost Group has reported data related to L-826,141 (**16**), a new CDP-840 derivative. L-826,141 provided *in vitro* PDE4A activity with an IC_{50} value at 2 nM. *In vitro*, L-826,141 blocked LPS-induced TNF-α release from human whole blood with IC_{50} value at 0.4 μM [260]. Historically, CDP-840 providing short half-lives in various species was further structurally modified in order to reduce the metabolism process. The synthetic efforts led to the identification of a first promising candidate, L-791,943 (**17**), which induced a 96% inhibition in a squirrel-monkey model of ascaris-induced bronchoconstriction after an oral administration of 3 mg/kg [261]. However, L-791,943 showed an excessively long half-life in numerous species. The elaboration of L-826,141, which is an optical isomer of L-791,943, gave an improved pharmacokinetic profile. In the squirrel-monkey antigen-induced bronchoconstriction model, L-826,141 was orally active showing 26-59% and 38-100% inhibition of the early and late phase responses in a dose range between 0.5 and 3 mg/kg [262-263]. In ferrets, L-826,141 was not emetic at 30 mg/kg *p.o.* [261-262]. L-826141 and its predessor, L-791943, were reported to be discontinued as clinical candidates [259].

The development of filamilast (**18**, WAY-PDA-641, Wyeth-Ayerst), a rolipram analog, was discontinued in phase 2.

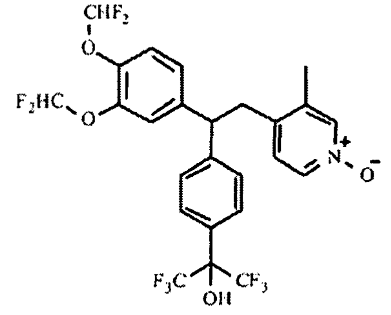
Celltech Group and Schering Plough developed D-4418 (**19**) up to clinical phase 2. D-4418 inhibited human PDE4 activity with an IC_{50} at 200 nM and LPS-induced TNF-α release with an IC_{50} at 160 nM. D-4418 was active *in vivo* in inhibiting TNF-α release in rats (ED_{50} = 10 mg/kg *p.o.*) and in blocking antigen-induced eosinophil accumulation into BAL in guinea-pig (40% inhibition at 10 mg/kg). In terms of safety profile, D-4418 induced no emetic or retching events at 60 mg/kg *p.o.* in ferrets and dogs. In phase I clinical trials, D-4418 showed good plasma exposure and was well tolerated at all doses [264]. Sch-351591 (D-4396, structure not reported) is structurally related to D-4418. This analog, being more potent than D-4418, is under development by Celltech Group (Chiroscience) and Schering Plough for the potential treatment of asthma. Phase I clinical trial has been underway since January 2000 [265].

Icos has recently undertaken a phase I trial with IC-485, a potential oral therapeutic for the treatment of inflammatory conditions [266]. This compound provided an IC_{50} from human recombinant PDE4 of 6.1 nM. The clinical study has been designed to assess the pharmacokinetic properties, safety and tolerability of IC-485 in healthy volunteers and is a double-blind, placebo-controlled, ascending oral dose study.

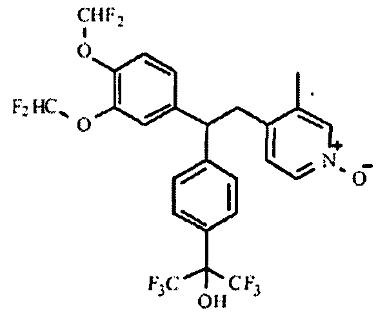
The therapeutic value of YM-976 (**21**, Yamanouchi) was assessed in phase I clinical trials. YM-976 at doses of 0.1 nM to 10 μM showed dose-related relaxation effect on tracheal preparations contracted with histamine and increases cAMP tissue content [267]. A general profile of YM-976 was recently reported [268]. YM-976 was the first compound having shown a dissociation of an anti-inflammatory activity (carrageenan-induced pleurisy) from emetic side effects in the



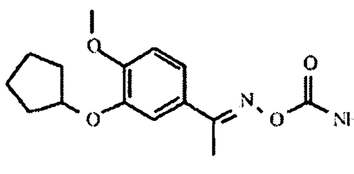
4 : CDP-840



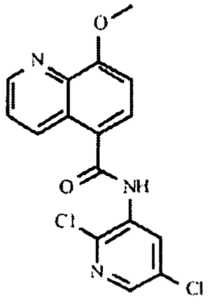
16 : L-791,943



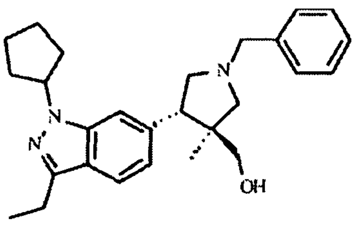
17 : L-826,141 = a L-791,943 optical isomer



18 : filamilast = WAY-PDA-641



19 : D-4418



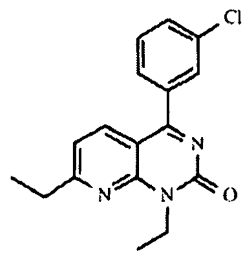
20 : IC-485

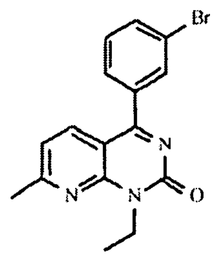
same animal species (ferrets). In comparison to various PDE4 inhibitors and particularly cilomilast, YM-976 provided a lower emetic liability and a broader safety profile. The mechanism of the lack of emetic potency remains to be identified. YM-58997 (**22**, Yamanouchi) is investigated under preclinical stage. No development was reported since 1998 for either Yamanouchi compounds.

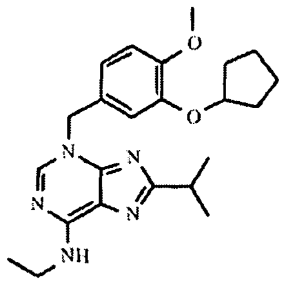
V-11294A (**23**, Napp), a xanthine derivative linked to a rolipram-based structure, entered into phase I clinical trials in 1997. In the ferret emesis model, the compound was safe up to 30 mg/kg *p.o.* keeping into account a bioavailability > 70% and plasma concentrations > 1μM. No emetic side effects were found in phase I clinical trial at doses up to 300 mg with a bioavailability > 50% and a plasma half life > 7 h [269]. Moreover, V-11294A produced a metabolite slightly less potent than itself [156]. In healthy volonteers, a 300 mg oral dose of V-11294A induced a reduction of TNF-α release from a LPS-induced *ex vivo* assay and a decrease of lymphocyte proliferation from a PHA induced model [270-271].

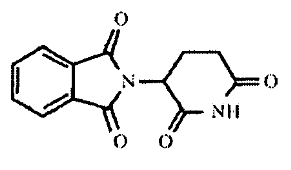
CDC-801 (**25**, Celgene), a thalidomid analog, is a lead of the first generation series of selective cytokine inhibitory drugs (SelCIDs). SelCID compounds are reported as potent inhibitors of TNF-α and PDE4 and presented as potent drugs for the treatment of inflammation, immune disorders, Crohn's disease and congestive heart failure. CDC-801 was evaluated in phase I clinical trials using escalating doses (50 to 1000 mg) and obtained promising results in a phase 2 pilot study for Crohn's disease [272]. In comparison to cilomilast, CDC-801 showed an improved therapeutic index versus emesis [273]. CD-998 (structure not reported, Celgene), a second-generation SelCID, was by far more than 100-fold more potent than CDC-801 against PDE4. CD-998 recently entered in early phase I trials and was found well tolerated. CC-7075 (**26**, Celgene) and CC-7085 (structure not reported), two other second-generation SelCIDs were equipotent and more potent than CDC-801 in inhibiting TNF-α and IFN-Gamma production [274]. CC-7085 was selected as being the back-up of CDC-801 [275].

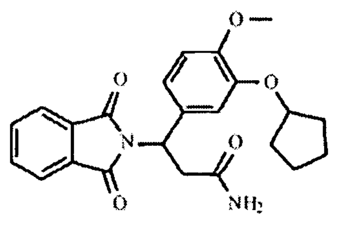
AWD-12-281 (**27**, ASTA Medica Arzneimittel GesmbH) is a 5-hydroxyindole derivative under preclinical investigations for the potential treatment of allergic asthma. Data showed anti-inflammatory effects of the compound with a very low emetic potential [276]. AWD-12-281 provided effect on mucus secretion in the mouse and LPS-induced neutrophilia in the rat and domestic pig [277]. As a model for COPD, the LPS-induced neutrophilia in rats was reduced by 37% and 69% at the dose of 30 mg/kg *p.o.* and 1 μg/kg after intrapulmonary administration [278]. A dose-dependent inhibition of allergen-induced late-phase eosinophilia was shown in sensitized rats (ID_{50} values at 7 μg/kg and 30 mg/kg respectively after intrapulmonary and oral administrations [279]. In a recent paper, RP-73401, rolipram, cilomilast and AWD-12-281 potently were shown to inhibit C5a-induced O_2-generation (IC_{50} = 0.03, 0.42, 0.55 and 0.86 μM, respectively), but failed to inhibit degranulation in the absence of adenylate cyclase activators such as histamine, salbutamol, prostaglandin E2 and forskolin. AWD 12-281 was the only one to block degranulation (IC_{50} = 16.2 μM) without any activation [280]. AWD-12-343 (**28**, ASTA Medica Arzneimittel GesmbH) emerged from a structure optimization process starting from AWD-12-281 and was selected for further preclinical investigations. This compound was potent with a nanomolar *in vitro* PDE4 activity (IC_{50} = 9 nM), highly selective versus PDE3, PDE5, and PDE7 (IC_{50}s > 1 μM) and provided a low affinity for the HARBS (IC_{50} = 313 nM) [281-282]. *In vitro*, AWD-12-343 inhibited TNF-α and GM-CSF release from human inflammatory cells (human polyp cells) with IC_{50} values of 0.22 and 0.26 μM, respectively. In bronchoalveolar lavage fluid (BALF), the

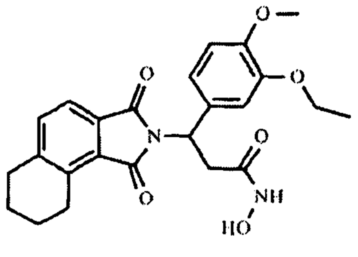

21 : YM-976

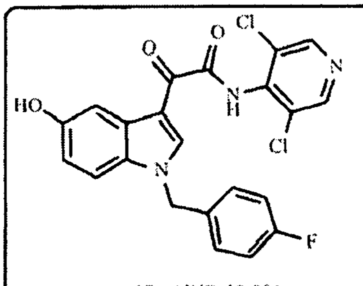

22 : YM-58997


23 : V-11294A

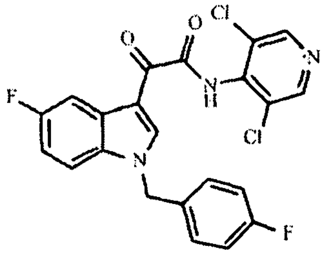

24 : thalidomide


25 : CDC-801


26 : CC-7075



27 : AWD-12-281



28 : AWD-12-343

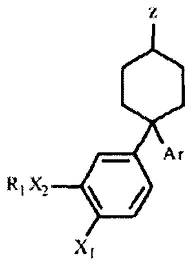
LPS-induced neutrophil recruitment reduced by 37% by AWD-12-281 at 30 mg/kg *p.o.* and by 67%, 61% and 90% after a 1 mg/kg oral administration of cilomilast, rolipram and AWD-12-343, respectively.

NEW CHEMICAL SERIES IN PDE4 AREA

Despite much progress in the development of PDE4, the search for new scaffolds is continuing with the aim to reduce the specific PDE4 side effects. This chapter attempts to give an overview of the global information coming from the scientific literature (papers and patents).

Cyclohexanes, Delta-Lactones or Lactames, Morpholines

A new series of 1,4-substituted cyclohexanes (**29**) structurally related to cilomilast was claimed by SmithKline Beecham (GlaxoSmithKline) [283]. The compounds were found to be PDE4 inhibitors and could be used to treat allergic and inflammatory diseases. They also inhibited the



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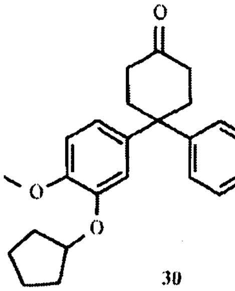

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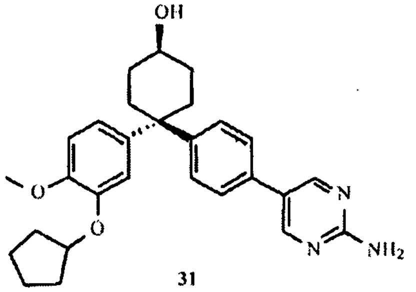

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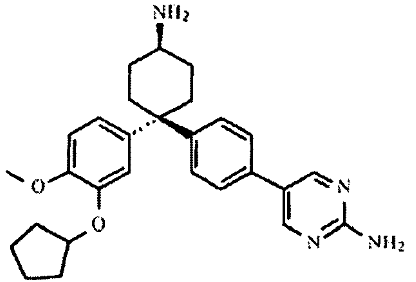

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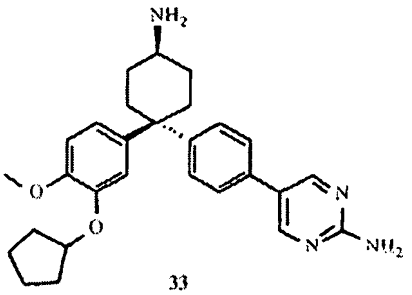

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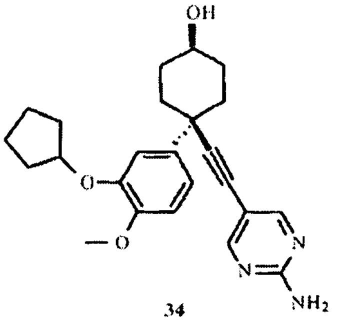


34

production of TNF-α. No biological data were reported and the compounds **30**, **31**, **32** and **33** were shown the most preferred compounds.

A novel construction of the quaternary C4 of such a cyclohexanol ring was reported for the synthesis of a cilomilast derivative, SB-222618 (**34**) [284].

Kyowa Hakko developed new heterocyclic derivatives (**35**) structurally related to cilomilast analogs [285]. (E)-3-[1-(8-Methoxy-2,3-dihydro-benzo[1,4]dioxin-5-yl)-4-oxo-cyclohexyl]-acrylic acid ethyl ester (**36**) inhibited 99% of human PDE4 at 1 μM. In EP-1110961, Kyowa Hakko described *cis*-4-cyano-4-(8-methoxy-1,4-benzodioxan-5-yl)cyclohexane carboxylic acid (**37**) showing 87% inhibition of the PDE4 enzyme at 1 μM [286].

The discovery of a new series of substituted γ-phenyl-Δ-lactones and their analogs including lactames as selective PDE4 inhibitors was described by Inflazyme [287]. (S)-5-(3-cyclopentyloxy-4-methoxy-phenyl)-3-(4-hydroxy-3-methoxy-benzyl)-tetrahydro-pyran-2-one (**38**) provided a PDE4 inhibition from U937 cell line with an IC_{50} value in a range from 0.1 to 1 μM. This compound inhibited the human neutrophil degranulation (via the inhibition of the reactive oxygen species in neutrophils) with an IC_{50} value lower than 1 μM and blocked the TNF-α production in a concanavalin A-stimulated human CD4+ T-cells with an IC_{50} between 2 and 20 μM. In this model, the compound showed an antioxidant activity *in vitro* and a Th1-inhibiting/Th2-sustaining profile. In addition, the compound displayed potent inhibitory activities in the resiniferitoxin-induced mouse ear edema after topical, intraperitoneal and oral administration with 98% inhibition at 50 mg/ear, 72% inhibition at 100 mg/kg and 45% inhibition at 10 mg/kg, respectively.

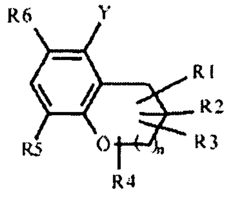
A new series of 2-phenylmorpholine derivatives (**39**) was presented by Nikken Chemicals [288]. Providing strong PDE4 inhibitory activity, these derivatives were reported for the treatment or prevention of inflammatory diseases such as asthma and dermatitis and autoimmune diseases such as multiple sclerosis and rheumatism. No biological data were reported.

Quinolines, Dihydroisoquinolines, Furoisoquinolines, Quinolinones, Phenanthridines, Naphthyridines, Naphthyridinones, Quinazolinones, Pirydopyrazines, Pyridopyrimidines and Pyridylnaphthalenes

In the past, quinoline derivatives were specifically claimed as PDE4 and TNF-α inhibitors by Darwin Discovery [289]. In 2000, the corresponding *N*-oxides (**40**) were described in a new patent [290]. No biological data were reported but these *N*-oxides (**40**), such as the exemplified 8-methoxy-2-trifluoromethylquinoline-5-carboxylic acid (3,5-dimethyl-1-oxypyridin-4-yl) amide (**41**), were stated to be selective PDE4 inhibitors useful for the treatment of inflammatory diseases.

Novel 8-aryl quinoline derivatives (**42**) were recently investigated by Merck Frost for their PDE4 and TNF-α inhibitory activities [291]. The IC_{50} values of the 42 exemplified compounds from human recombinant PDE4A enzyme were stated to be 0.14 nM to 10.2 nM. In an ovalbumin-challenged guinea-pig model, these candidates, at 0.001 to 10 mg/kg *i.p.* or *p.o.* twice a day, induced a significant reduction of the production of eosinophils and leukocytes.

Recently, Sanofi-Synthelabo reported the syntheses of novel isoquinoline derivatives (**44**) as PDE4 inhibitors that could be suitable for the treatment of for instance asthma, COPD and rheumatoid arthritis [292-293]. The PDE4 IC_{50} of isoquinolines **45**, **46** and **47** were in a nanomolar range. The compounds were selective versus PDE2, PDE3, and PDE5.



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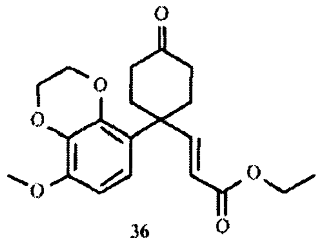

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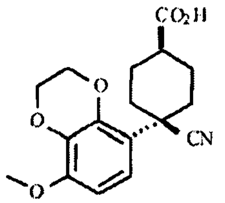

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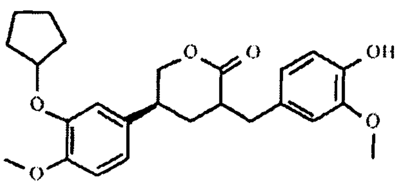

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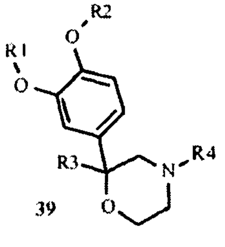

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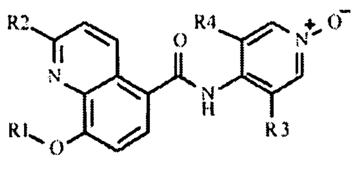

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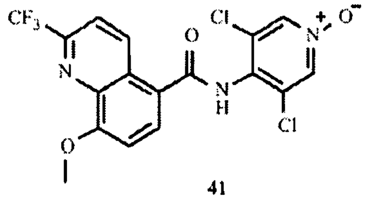

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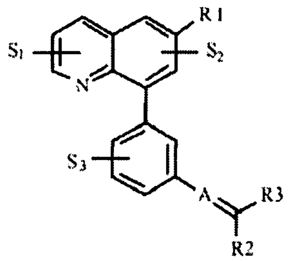

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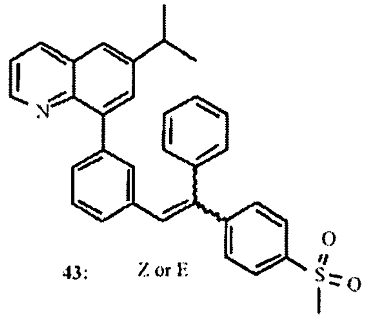

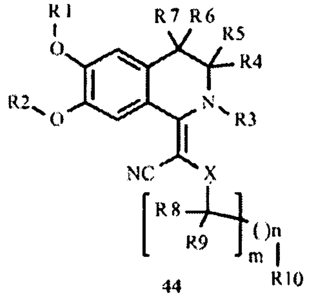

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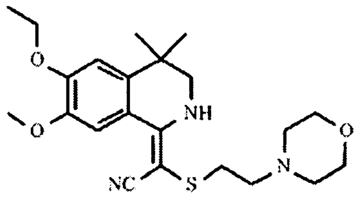
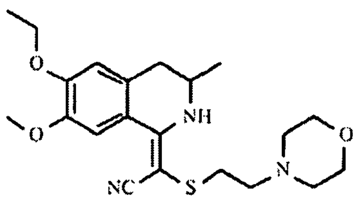
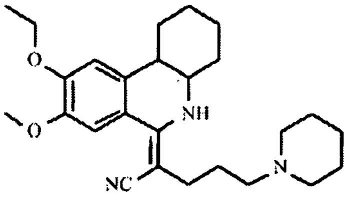


45
PDE4 IC_{50} = 2.7 nM



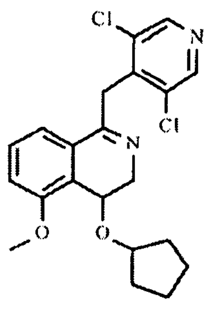
46
PDE4 IC_{50} = 8.8 nM



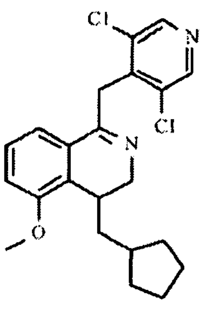
47
PDE4 IC_{50} = 6 nM

In 1999, novel of 3,4-dihydroisoquinolines and 2,3-dihydroquinolin-4-ones were disclosed in Zambon patents in which **48** was described as one of the most potent PDE4 inhibitors *in vitro* with an IC_{50} value at 148 nM [294]. Later on, another series of dihydroisoquinolines was synthesized [295]. The cyclopentylmethylene analog (**49**) of the compound **48** provided an improved *in vitro* PDE4 activity with an IC_{50} at 35.9 nM.

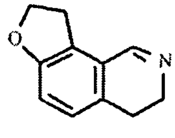
Takeda investigated furoisoquinoline derivatives as PDE4 inhibitors for treating and preventing diseases caused by inflammation [296]. The compounds showing the partial



48
PDE4 IC_{50} = 148 nM



49
PDE4 IC_{50} = 35.9 nM



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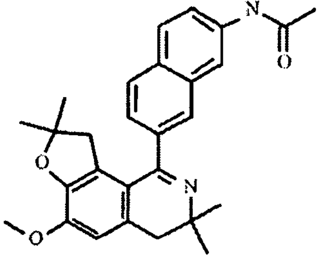

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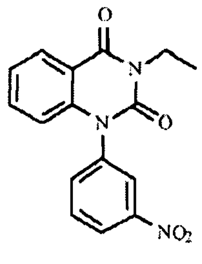
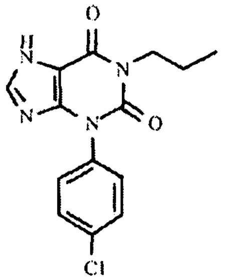
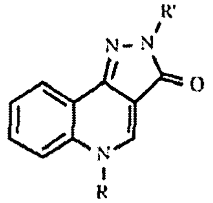


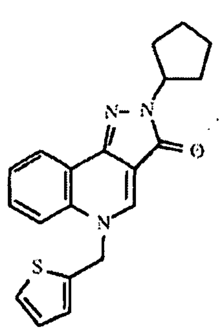
52 - Nitraquazone



9 - Arofylline



53 - DHPQ



54

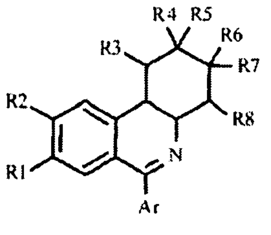
furoisoquinoline structure (**50**) were disclosed as PDE4 inhibitors. At 1 mg/kg *i.v.*, the compound **51** prevented 59% of constriction in an ovalbumin-induced bronchial constriction model in New Zealand white rabbits.

Almirall Prosderma group specified a new series of 2,5-dihydropyrazolo[4,3-*c*]quinolin-3-one (**DHPQ**) derivatives as PDE4 inhibitors [297]. These compounds were designed using pharmacophores coming from nitraquizone (**52**) and arofylline (**9**). A lipophilic group at *N2* position was found to be required for PDE4 activity. Compound **54**, with a 2-thienylmethyl group at *N5* position and a cyclopentyl at *N2* position showed a PDE4 activity similar to that of rolipram and an improvement of HARBS/PDE4 ratio > 100 fold. From *in vivo* guinea-pig models, the compound **54** at 10 mg/kg *p.o.* provided 62% inhibition of eosinophil infiltration in BAL and an inhibition of histamine-induced bronchoconstriction with an ED_{50} value at 83 µg/kg after *i.v.* administration. The minimum emetic dose *i.v.* in dogs of that compound was >3 mg/kg.

In 2001, Byk Gulden reported several novel phenanthridine series (**55**) as selective PDE4 inhibitors [298-300]. The *in vitro* PDE4 activities (-logIC_{50} values) of the phenanthridines (**55**) were ranged from 6.76 to 8.87. Among the most potent derivatives, compounds **56**, **57** and **58**, which are *cis*-enantiomers with negative optical rotation data, provided submicromolar IC_{50} values.

Within the same time period, Byk Gulden published three patents related to the PDE4 activity of novel phenanthridine-*N*-oxide derivatives (compounds **59**) [301-303]. The *in vitro* activities (-logIC_{50} values) of compounds **60**, **61** and **62** were 6.73, 6.29, and 6.09 respectively. Again, these compounds were reported to be the (-)-*cis*-enantiomers.

Novartis recently described the synthesis and SAR of 6,8-disubstituted-1,7-naphthyridines (**63**) that are potent PDE4D subtype-selective inhibitors [304]. The most potent compounds (**64-66**) showed activity in an *in vivo* model of allergic asthma. An appropriate substitution at 8-position (NO_2, Cl, CN) was reported to be important for good PDE4A-D inhibitory activity. Then, the compounds with a small group, such as H or NH_2, at 6-position provided a weak micromolar PDE4A-D activity. The introduction of bulkier groups at this position increased the inhibitory potency toward PDE4B and mostly PDE4D. The benzoic acid derivative (**64**) was the most active compound in this series with a PDE4D activity value at 1 nM. This analog was 49-, 68-, and 88-fold more potent on PDE4D than PDE4B, PDE4A and PDE4C, respectively and was 79-fold



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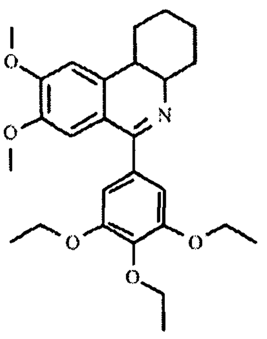
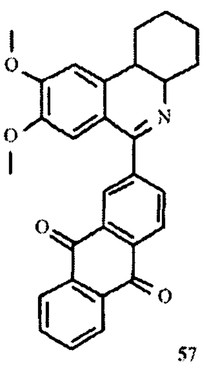
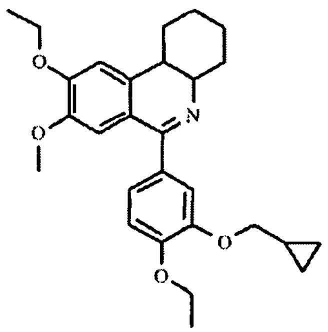


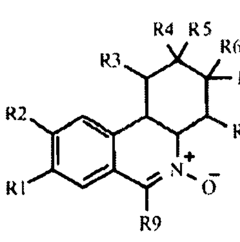
56
-logIC_{50} = 7.81



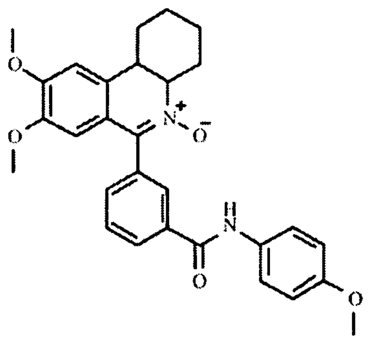
57
-logIC_{50} = 8.00



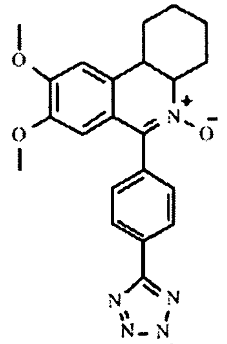
58
-logIC_{50} = 8.87



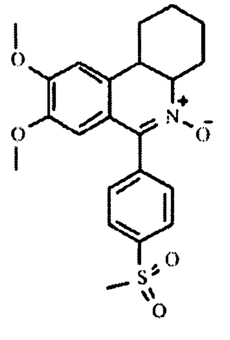
59



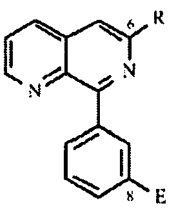
60
PDE4 -logIC$_{50}$ = 6.73



61
PDE4 -logIC$_{50}$ - 6.29



62
PDE4 -logIC$_{50}$ = 6.09



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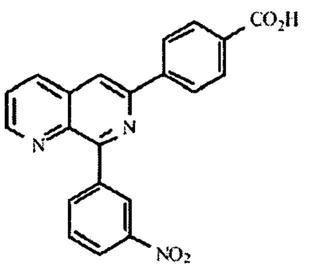

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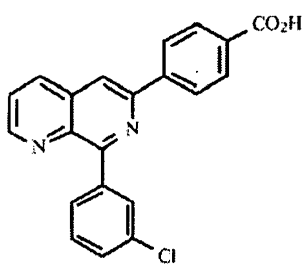

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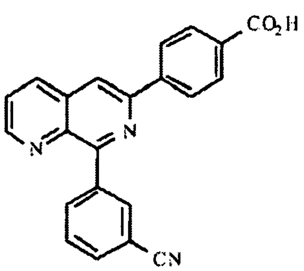
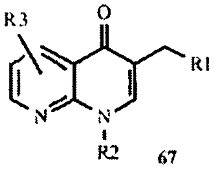


66

more potent on PDE4D than cilomilast. In a model of allergic asthma in Brown-Norway rats, **64** administered at 1 mg/kg *p.o.* decreased by more than 50% the influx of eosinophils, T-cells, and neutrophils in BAL. Finally, at 1 mg/kg *p.o.*, the benzoic acids (**64**, **65** and **66**) were more active than cilomilast in this oral antigen-induced pulmonary eosinophilia rat model.

Pfizer described a new series of 1,8-naphthyridin-4[1*H*]-ones (**67**) [305]. Such derivatives were reported to selectively inhibit the PDE4D isozyme and to block the pulmonary eosinophil infiltration in monkey BAL after subcutaneous administration.

A series of 1,8-naphthyridin-2-one derivatives was identified by Grelan (compounds **70** such as **71**) and Yamanouchi (compounds **72** such as **73**) for providing new PDE4 inhibitors useful for treating inflammatory and autoimmune diseases [306-307]. 1-(3-Nitro-phenyl)-3-(3-pyridin-3-yl-propyl)-1*H*-[1,8] naphthyridin-2-one (**71**) inhibited the reduction in bronchial capacity by 95% at 3 mg/kg after oral administration. 3-[4-(3-Chloro-phenyl)-1-ethyl-7-methyl-2-



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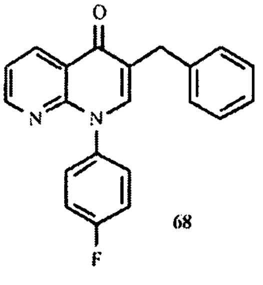

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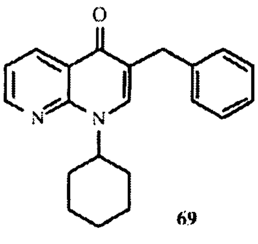

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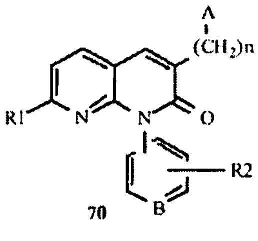

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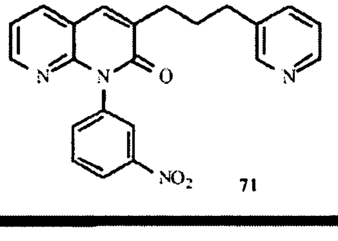

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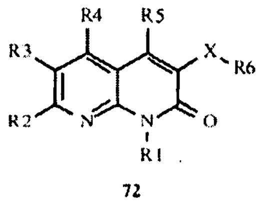

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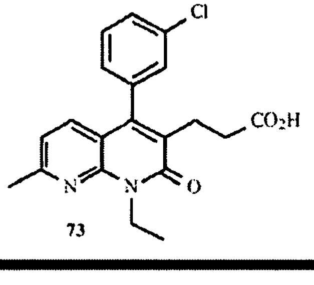

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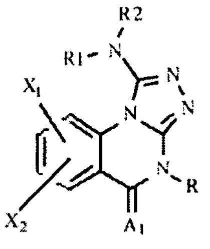

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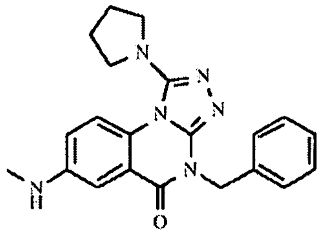
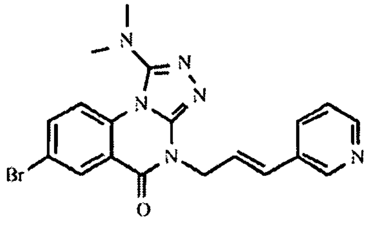


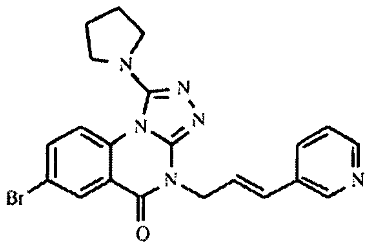
75

PDE4 IC_{50} = 0.00027 µM



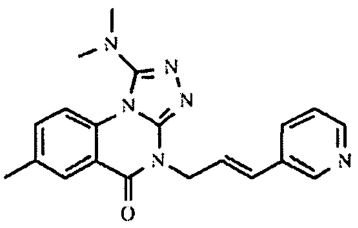
77

PDE4 IC_{50} = 0.079 µM



76

PDE4 IC_{50} = 0.014 µM



78

PDE4 IC_{50} = 0.077 µM

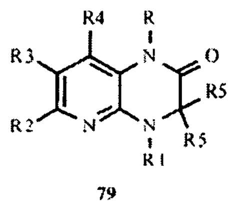
oxo-1, 2-dihydro-[1,8] naphthyridin-3-yl]-propionic acid (**73**) displayed a PDE4 IC_{50} of 0.011 µM.

An extended series of triazolo quinolin-ones or -thiones (**74**) was reported by Parke Davis (Pfizer) [308]. These compounds were found to be selective PDE4 inhibitors. In addition, they inhibited TNF-α release. The compounds provided PDE4 IC_{50}s between 0.00027 and 2.69 µM. Several derivatives inhibited TNF-α release from human leukocytes: the compounds **77** and **78** showed IC_{50}s at 3.4 and 8.1 µM, respectively. Moreover, various compounds were evaluated *in vivo* in a LPS-induced TNF-α model in rats. The compounds **77** and **78** provided 98% and 94% inhibition at the oral dose of 10 mg/kg.

Novel 1,4-disubstituted pyridopyrazine derivatives (**79**) synthesized by Yamanouchi were claimed PDE4 inhibitors [309]. No biological data were presented and the specified compound was 1-(3-bromophenyl)-4-ethyl-6-methylpyrido [2,3,b]pyrazine-2,3(1*H*, 4*H*)-dione (**80**).

In the past, pyridopyrimidine moiety was reported for exhibiting PDE4 inhibitory activity. Several pyridopyrimidine derivatives (**81**) were also disclosed as PDE4 inhibitors by Yamanouchi [310]. These compounds were stated to be of use to treating asthma. No biological data were reported and one compound, 5-(3-bromophenyl)-2-methyl-8,9-dihydroimidazo[1,2-*a*]pyrido[3,2-*e*]pyrimidine (**82**) was specified.

Very recently, the syntheses and *in vitro* evaluation of a new pyridopyrimidine-2,4-dione series (**83**) bearing substituents on the pyridine ring were described [311]. All the C-4 substituted pyridopyrimidine analogs (**85**) provided more potent inhibitory activity than the corresponding C-3 substituted compounds (**84**) except for the benzyl



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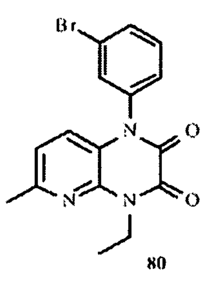

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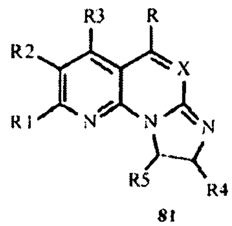

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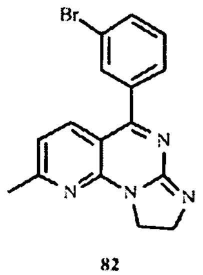

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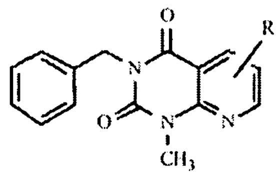

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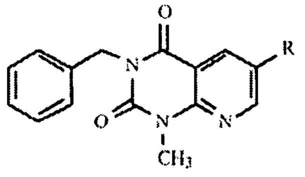
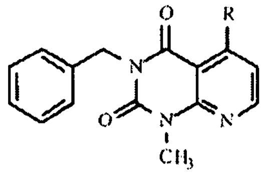


84: C-3 analogs



85 : C-4 analogs

R = O-nBu, CN, CO_2Et, Ph, 4-Cl-Ph, 4-F-Ph, 4-EtO-Ph,

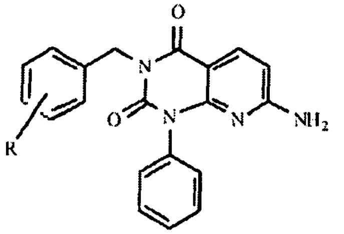
2-F-Ph, 2-CF_3-Ph, 3-NO_2-Ph, 3-CF_3-Ph, CH_2Ph

derivatives. The derivative, with a benzyl group at the C-3 position, was 33 fold more active than its C-4 substituted analog. Moreover, it was equipotent to cilomilast on LPDE4 (IC_{50} at 0.11 µM) and showed a more promising ratio HPDE4/LPDE4 than cilomilast (212.73 instead of 1.64 for cilomilast).

Nippon Zoki also disclosed another series of pyridopyrimidines (**86**) for the treatment of dermatitis [312]. 7-Amino-3-benzyl-1, 2, 3,4-tetrahydro-1-phenylpyrido[2,3-*d*] pyrimidin-2,4-dione (**87**) was the preferred candidate. It was reported to provide a selective PDE4 inhibition versus PDE2, PDE3, and PDE5 with 82% inhibition at 100 µM.

Phthalazines, Phthalazinones, Pyridazones, and Pyridazines

Zambon group prepared some rigid analogs of RP-73401 (**3**) to gather information on the conformation that could be adopted by the phenyl ring in the binding site [313]. Therefore, they designed new cyclic derivatives in which the carbonyl group was substituted by a π-bond involved in a pyridazine nucleus which was also supposed to increase the polarity of the new RP-73401 analogs (**88**). The activities of most of phthalazines (**88**) with PDE4 IC_{50} values < 100 nM showed that, for the RP-73401 related compounds, a planar dihedral angle between the aromatic moiety and the linker was allowed. The phthalazines **88** with a cyclopentyloxy group on R1 were reported to be the preferred compounds in comparison to the derivatives with a cyclopentyloxy on R2. The phthalazine (**89**) displayed a PDE4 IC_{50} value at 53 nM and provided improved safety margins versus acid secretion and emesis against several PDE4 standards. The phthalazine (**89**) diplayed a minimal emetic dose in dogs > 10 mg/kg *i.v.* whereas cilomilast induced emesis through the same route of administration with an ED_{50} at 10 mg/kg. The phthalazine (**90**) was one of the most potent derivative *in vitro* with an IC_{50} value at 8 nM [314]. Based on the decreased potency of the phthalazine (**89**) compared to the open reference compound RP-73401 (PDE4 IC_{50} values at 53 nM and 1 nM, respectively), Zambon group initiated the synthesis of a novel series of phthalazines (**91**) with substituents in position 4 of the phthalazine nucleus [315]. The aim of the study was to avoid a possible unfavorable interaction between the cyclopentyloxy and the *peri-*



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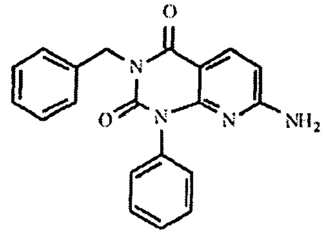

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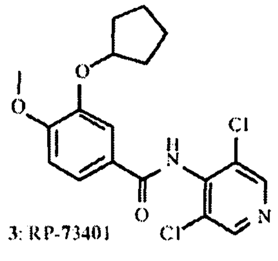
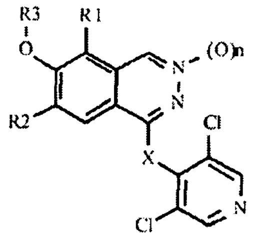


3: RP-73401



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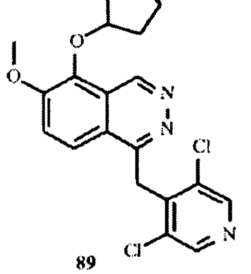

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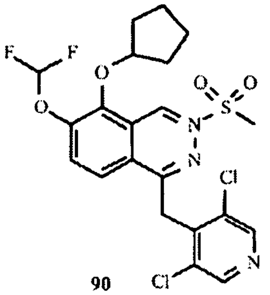

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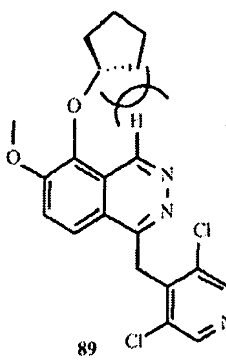

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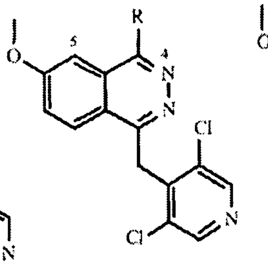

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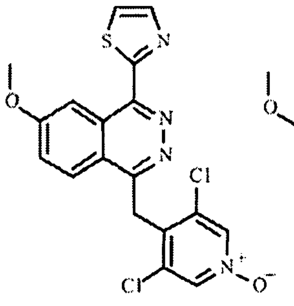

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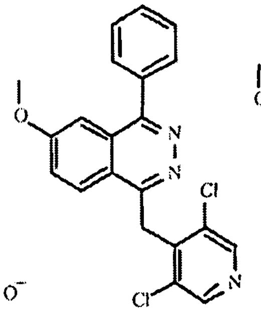

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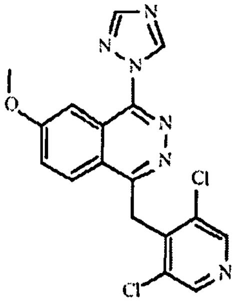


94

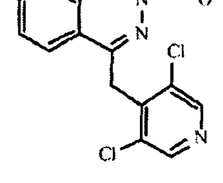
hydrogen in position 4 of the phthalazine nucleus (**89**) and to evaluate new derivatives (**91**) with various substituents at position 4 instead of position 5.

Potent PDE4 inhibitory activity was shown with various phthalazines **91**. Only one derivative, the pyridine *N*-oxyde phthalazine **92**, was in a same range of activity than RP-73401 with *in vitro* PDE4 IC_{50} values at 4 nM instead of 1 nM for RP-73401. Most compounds showed *in vitro* PDE4 activities similar to those found with the most active phthalazines **88** (IC_{50}s between 30 and 250 nM). While the structure-activity relationships of phthalazines **91** remained to be explained, several substituents with various physicochemical parameters led to potent new candidates with potent PDE4 activity and whole cell efficacy. Eosinophilia in guinea-pigs and side effects of two phthalazines, **92** (PDE4 IC_{50} = 37 nM) and **93** (PDE4 IC_{50} = 241 nM) were evaluated. The two compounds were found more active than cilomilast in inhibiting eosinophil infiltration. Finally, acid secretion increase in isolated rat stomach and emesis in dog were reduced in comparison with those found with cilomilast.

The phthalazine (**95**) with a methoxycarbonyl group and with an *in vitro* PDE4 IC_{50} value at 72 nM was specifically claimed [316]. In addition, several phthalizones were reported: the 3-methylsulphonyl phthalazinone **96** provided a PDE4 IC_{50} at 36 nM.

In a recent patent, Zambon disclosed a series of tricyclic phthalazines and exemplified one compound tested, the tricyclic phthalazine **97** which displayed an *in vitro* PDE4 IC_{50} at 207 nM [317].

Novel PDE4 phthalazinones were recently described by Byk Gulden in two papers. A goal of Byk Gulden was to discover new lead structures (**99**) for selective PDE4 or dual PDE3/4 inhibitors coming from a combination between the pyridazone zardaverine (**5**, a wellknown PDE3/4 inhibitor with a PDE4 pIC_{50} = 6.80 and a PDE3 pIC_{50} = 6.24) and a selective PDE4 pyridopyridazone (**98**, PDE4 pIC_{50} = 8.30) [318]. Therefore SAR studies were applied on a series of 6-(3,4-dimethoxyphenyl)-4,5-dihydro-*2H*-pyridazin-3-ones (**100**) and a series of 4-(3,4-dimethoxyphenyl)-*2H*-phthalazin-1-ones (**101**). The authors described various pyridazinones and a novel series of phthalazinones with potent PDE4 inhibitory potency. Most compounds were found selective PDE4 inhibitors. However, as it was already reported in the literature, SAR studies showed for pyridazinone derivatives with a non substituted amide moiety (R3 = H) the importance of a methyl group at the 5-position for potent PDE3 inhibition. In both series, the *N*-substitution was preferred for a PDE4 activity. The *cis*-4a,5,6,7,8,8a-hexa- and *cis*-4a,5,8,8a-tetrahydrophthalazi-nones (compounds **101** with rings C and D, respectively) were the most potent derivatives with pIC_{50} at 8.4 and 8.1, respectively. Molecular modeling studies showed that the *cis*-fused cyclohex(a)ene ring compounds do not overlap with the other fused-ring phthalazinones: the *cis*-fused cyclohex(a)enes fill a different region of space than the other fused rings which are more or less coplanar with the heterocyclic cycle. Then, the synthesis and structure-activity



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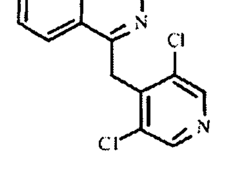

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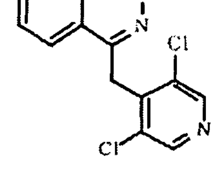

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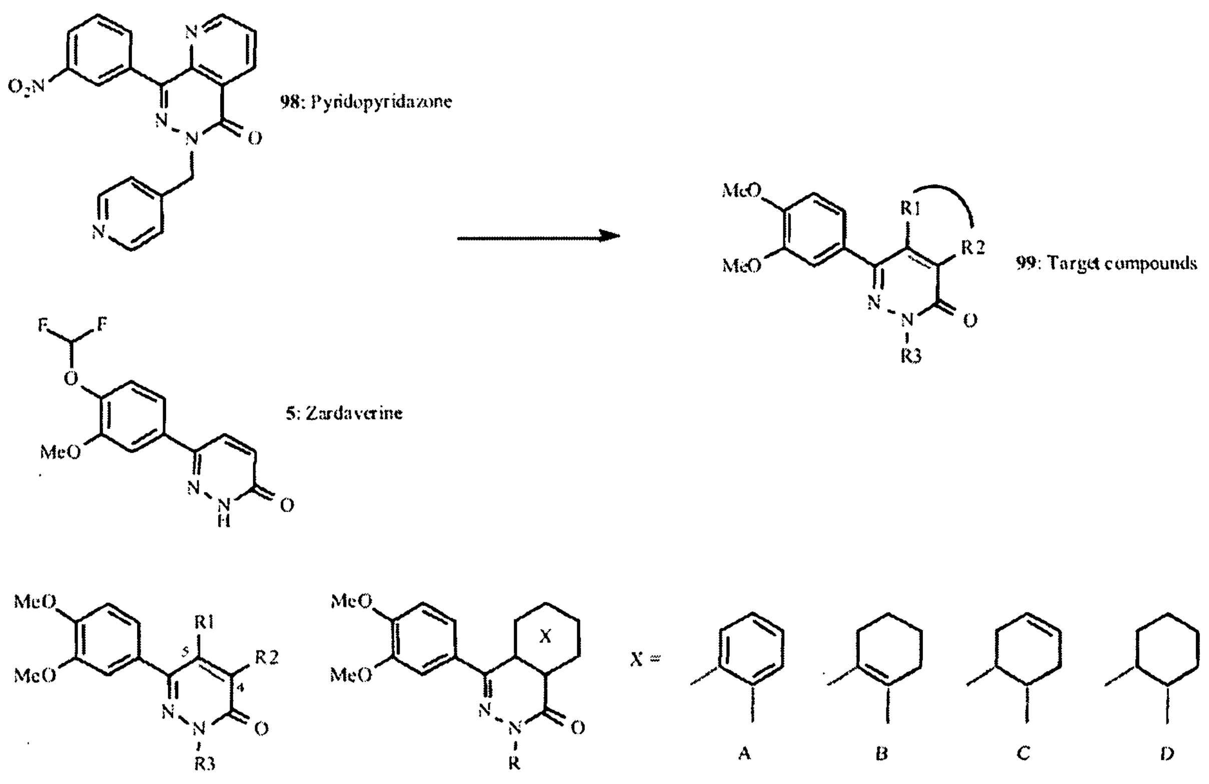


98: Pyridopyridazone

99: Target compounds

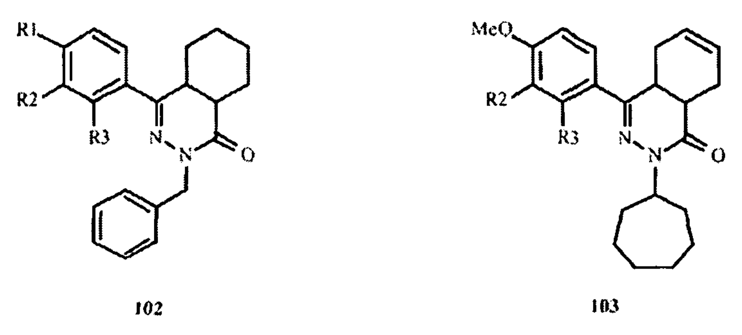
5: Zardaverine

100: pyridazinone series

101: phthalazinone series

relationships of new 4-aryl-substituted *cis*-4a,5,6,7,8,8a-hexa (**102**) and *cis*-4a,5,8,8a-tetrahydrophthalazinone (**103**) series were reported [319]. Several new derivatives were more potent than rolipram and cilomilast in term of *in vitro* PDE4 activity. In both series, the 4-(3,4-dialkoxyphenyl) group was found crucial for potent PDE4 inhibition and the SAR study in this area was generally found similar to those evaluated in the past with 3,4-dialkoxyphenyl moiety of various rolipram-like PDE4 inhibitors such as RP-73401. However, in contrast to the inhibitors structurally related to rolipram, these phthalazinone series were not found affected by the substitution of the 3-methoxy group with larger hydrophobic moieties.

Very recently, Byk Gulden reported novel series of PDE4 phthalazinones (compounds **104** and **111**) [320-321]. In the first patent WO-0130766, the *in vitro* PDE4 activity of 6 *cis*-derivatives (compounds **105-110**) was presented: the -logIC$_{50}$ values were ranged from 7.81 to 9.71. The second patent WO-0130777, was related to new tetrahydrothiopyranyl phthalazinone derivatives (compounds **111**): the -logIC$_{50}$ values of 8 compounds, from 8.02 to



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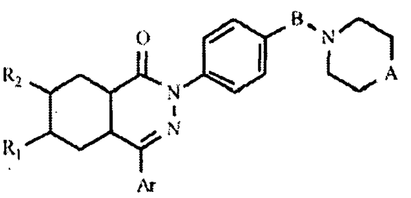

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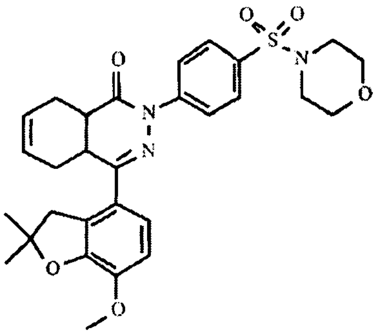
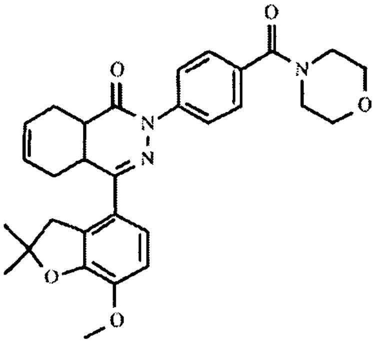
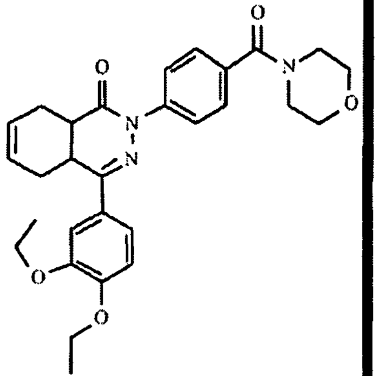


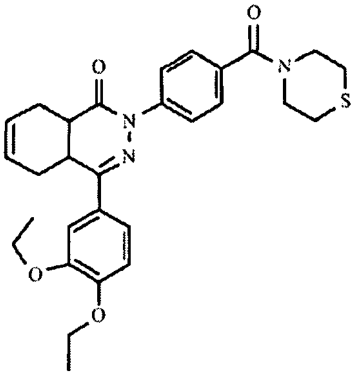
105: PDE4 -logIC$_{50}$ = 9.71



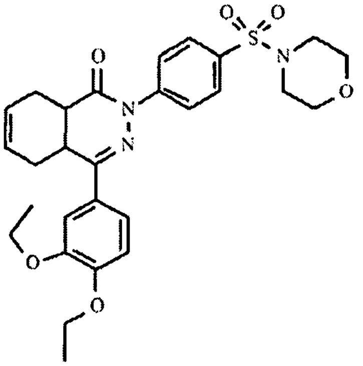
106: PDE4 -logIC$_{50}$ = 8.77



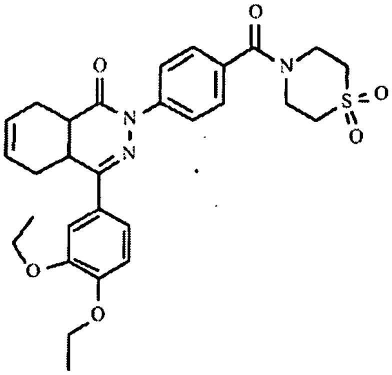
107: PDE4 -logIC$_{50}$ = 7.99



108: PDE4 -logIC$_{50}$ = 7.82



109: PDE4 -logIC$_{50}$ = 9.22

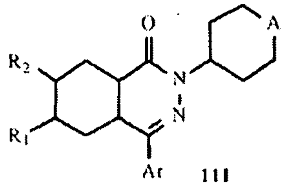


110: PDE4 -logIC$_{50}$ = 7.81

9.43, were reported. The 4S, 8R-*cis* derivatives seemed to be the most potent PDE4 inhibitors (comparison between **114** and **115**). The aryl substitution with the 3-cyclopentyloxy-4-methoxyphenyl group, structurally-related to rolipram, or the 2,2-dimethyl-7-methoxybenzofuran-4-yl moiety increased the *in vitro* PDE4 potency in comparison to the corresponding dimethoxy and diethoxy derivatives (comparison between **119**, **113**, **118** and **114**).

The synthesis of new series of pyridazine derivatives (**120**, **122**, and **123**) were described by E Merck as potent PDE4 inhibitors that could be used to treat allergic, inflammatory and autoimmune diseases [322-326]. No biological data were reported for the new pyridazines (**120**), such as the 4-chloro-*N*-{3-[3-(3-ethoxy-4-methoxy-phenyl)-5,6-dihydro-2*H*-pyridazine-1-carbonyl]-phenyl}-ben-zamide (**121**), and for the new benzoylpyridazines (**122** and **123**).

In 1996, a new structural class of 1-arylnaphthalene derivatives was reported as showing a selective PDE4 inhibitory activity. Recently, Tanabe designed compounds (**126**) as hybrids of the lead T-440 (**124**) and compound **125** [327]. An efficient synthesis of T-440 was described in 2000 [328]. Among the novel 1-pyridylnaphthlene compounds (**126**), T-2585.HCl (**127**) was a promising PDE4 selective candidate with a PDE4 IC$_{50}$ value at 0.13 nM. This compound provided good antispasmogenic activities in guinea-pig, intravenously for the reduction of the antigen-induced bronchoconstriction (ED$_{50}$ = 0.033 mg/kg) and intraduodenally for the diminution of the histamine-induced



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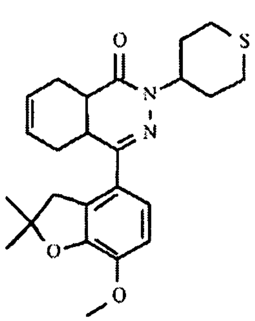
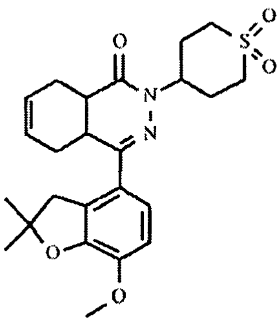
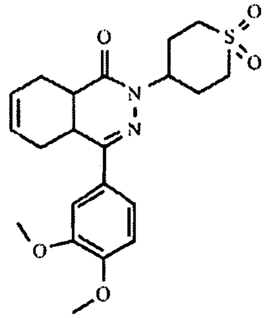


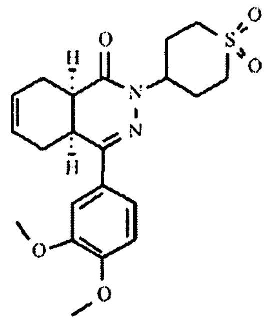
112: PDE4 -logIC$_{50}$ = 9.34



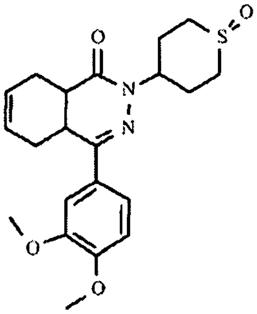
113: PDE4 -logIC$_{50}$ = 9.00



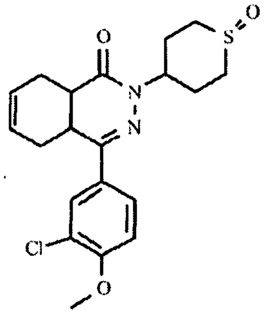
114: PDE4 -logIC$_{50}$ = 8.45



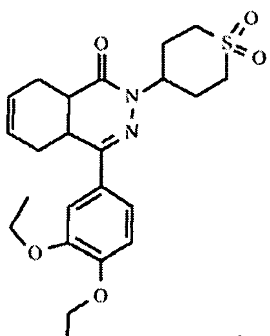
115: PDE4 -logIC$_{50}$ = 9.20



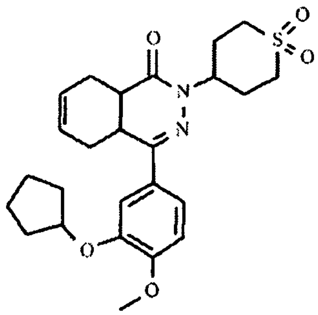
116: PDE4 -logIC$_{50}$ = 8.51



117: PDE4 -logIC$_{50}$ = 8.20

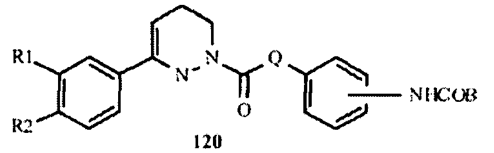


118: PDE4 -logIC$_{50}$ = 8.02

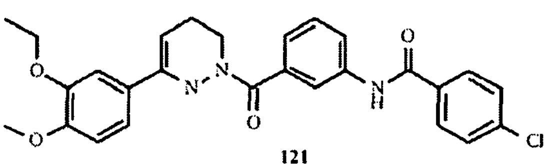


119: PDE4 -logIC$_{50}$ = 9.43

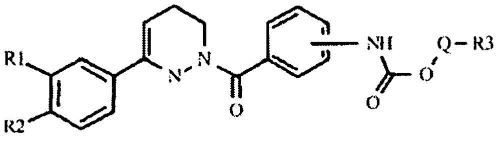
brochoconstriction (ED$_{50}$ = 0.063 mg/kg). Weak cardiovascular effects were observed during the experiments. T-2585 was less emetic than RP-73401 (**3**) after oral administration in ferrets and intravenous administration in dogs: emetic events appeared in ferrets at 30 mg/kg *p.o.* instead of 1 mg/kg for RP-73401 and in dogs at 1 mg/kg *i.v.* instead of 0.3 mg/kg for RP-73401. In JP-2000063275, Tanabe disclosed 52 new medicinal compounds with a PDE4 inhibiting profile [329]. One of the specified candidate, the 4-(3-hydroxymethyl-6,7-dimethoxyisoquinolin-1-yl)-2-(4-methyl-1-oxo-2*H*-phthalazin-2-yl)pyridine (**128**) displayed 97% inhibition of bronchoconstriction at 1 mg/kg in a guinea-pig histamine-induced asthma model.



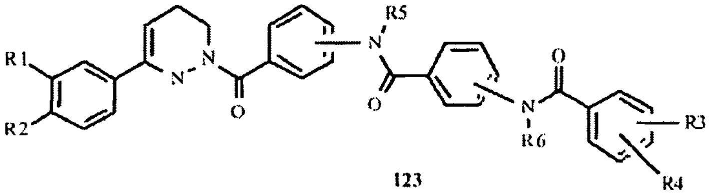
120



121



122



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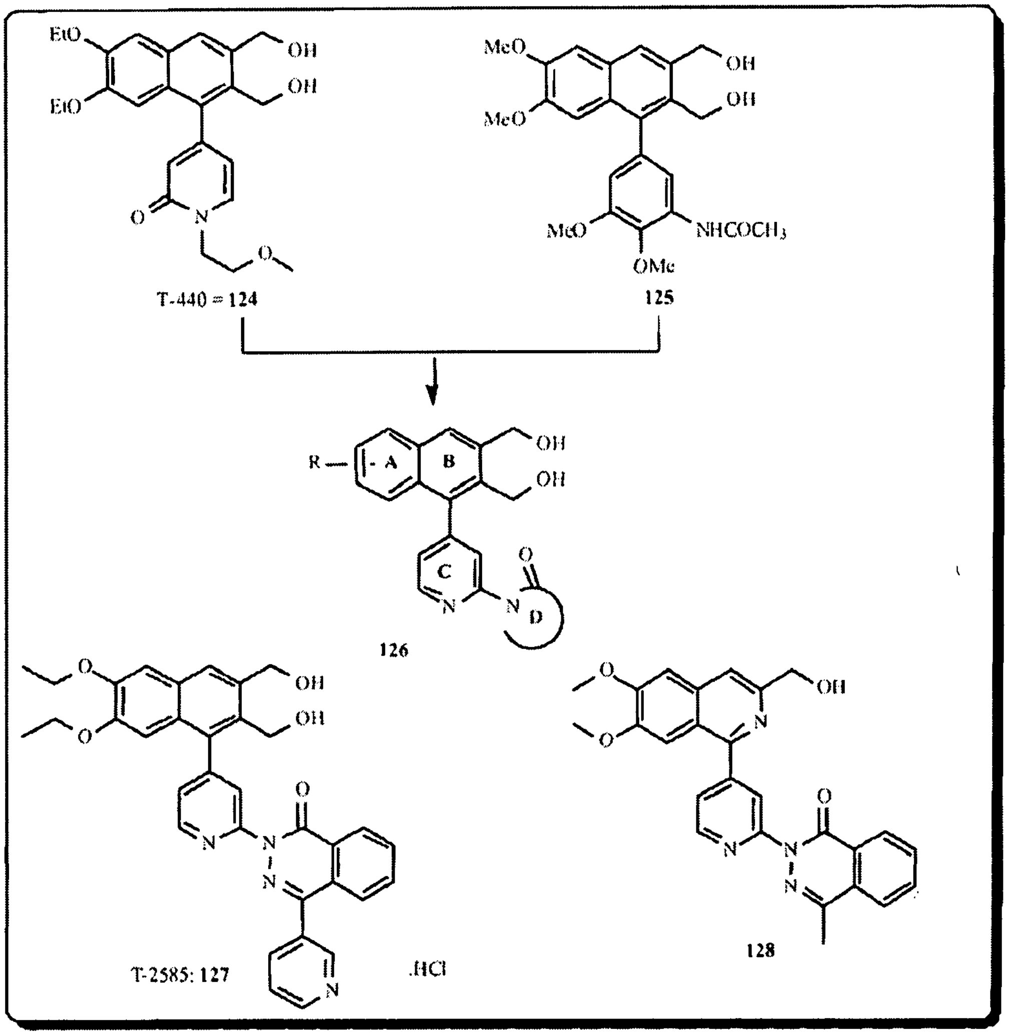
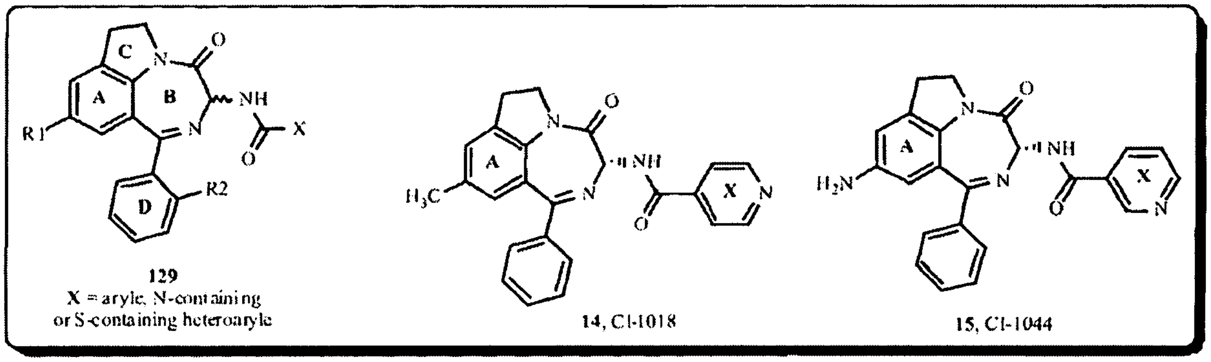
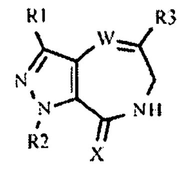


Diazepinoindoles and Pyrazolodiazepines

Parke Davis (Pfizer) reported the discovery of a novel series of benzodiazepine derivatives (**129**) as PDE4 inhibitors [330]. Regarding the cycle **C**, the 5-membered ring was preferred for an effective *in vitro* PDE4 activity. In comparison to the derivatives with a 6-membered ring or no rings **C**, the 5-membered ring analogs are supposed to increase the level of structural rigidity and to adopt only 2 low-energy conformations. The active isomer was found to be of R configuration. Classical SAR studies on rings **A**, **D** and **X** were carried out and a single compound, CI-1018 (**14**), was selected for further pharmacological studies [248-251, and see CI-1018 in chapter on clinical development of PDE4 inhibitors].

Later on, starting from CI-1018 (**14**), a SAR optimization was undertaken to evaluate the effects of substitutions on both heteroaromatic rings **A** and **X** and a novel series of aminodiazepinoindoles was described [253]. In comparison to the derivatives with a methyl or a methoxy group at the 9-position (R1), the primary amino analogs





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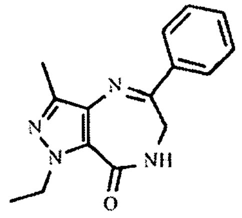
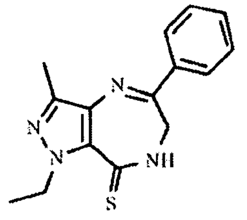
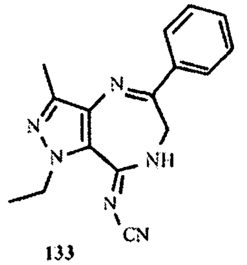


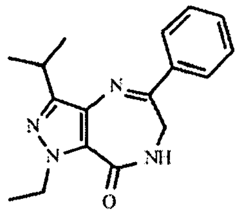
131
PDE4 IC_{50} = 0.088 μM



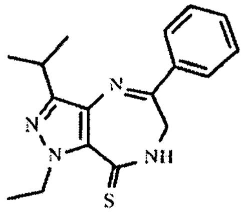
132
PDE4 IC_{50} = 0.06 μM



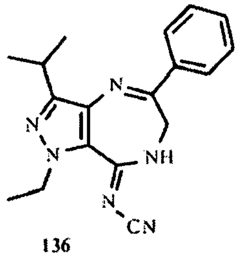
133
PDE4 IC_{50} = 0.0017 μM



134
PDE4 IC_{50} = 0.005 μM



135
PDE4 IC_{50} = 0.001 μM



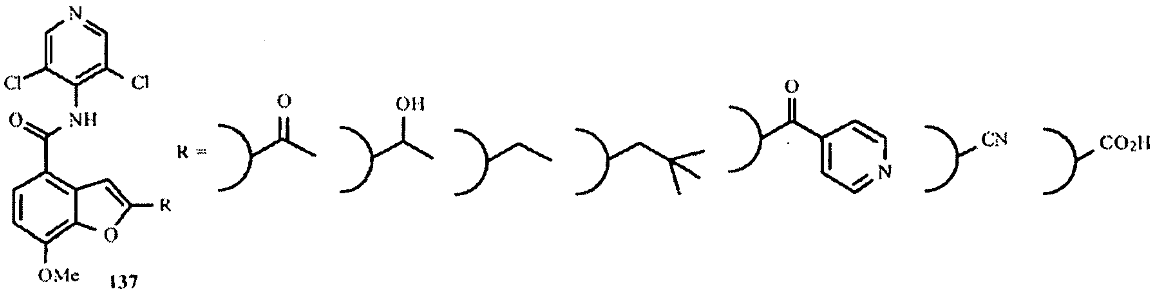
136
PDE4 IC_{50} = 0.00012 μM

were found to be the most potent PDE4 inhibitors with submicromolar PDE4 IC_{50}s. In addition, the NH_2 group was preferred to improve the PDE4 selectivity versus PDE1, PDE3 and PDE5. The mono- and disubstituted amino derivatives reduced the PDE4 selectivity as well. The SAR studies on X-ring remained unclear and several heteroaromatic rings or substituted phenyl moieties were found able to improve *in vitro* PDE4 activity. The improvement of the NH_2 introduction at 9-position was particularly significant through the data obtained *in vitro* (TNF-α inhibition from hWB) but also *in vivo* in rats (inhibition of TNF-α production and BAL eosinophil recruitment). In these different models, most amino derivatives were by far more active than their corresponding methyl analogs and also more potent than cilomilast. Finally, several selected compounds showed improved therapeutic index between efficacy and emesis. CI-1044 (**15**) was selected for further development investigations [254, 256-257, and see CI-1044 in chapter on Clinical Development of PDE4 inhibitors]. The combination of three different techniques of QSAR analysis was applied to the novel series of benzodiazepines not only for improving the understanding of the structural basis for the benzodiazepine affinity to the catalytic center of the PDE4 enzyme but also for providing a new approach in the design of PDE4 inhibitors [331].

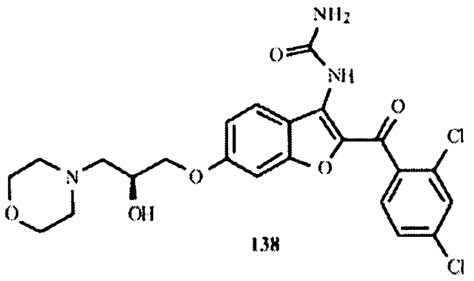
Pfizer recently disclosed a series of novel substituted pyrazolodiazepines (**130**) [332-333]. Several derivatives showed a promising inhibitory potency from human PDE4 enzyme. Some interesting compounds (**131-136**) were from 10 to 7000 fold more active than rolipram (PDE4 IC_{50} = 0.86 μM). The substitution of the oxygen atom of the amide moiety by a sulfur atom led to compounds 1.4 to 5 folds more active. In addition, in the same area, the introduction of a cyanamide group drastically improved the *in vitro* PDE4 activity, ~40 to 50 folds in comparison to the amido derivative activity. It was note worthy that bulkier hydrophobic groups at R1-position increased the *in vitro* PDE4 activity.

Benzofurans, Benzoxazoles, Benzothiazoles, Indoles, Pyrazoles, Pyrrolidines, Pyrrolidinones and Thiazoles

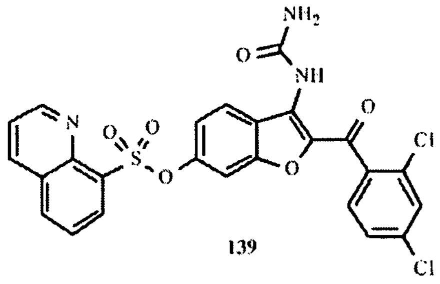
In the past, various benzofuran derivatives were described as potent PDE4 inhibitors. The use of benzofuran nucleus was reported by Rhône-Poulenc Rorer for the replacement of



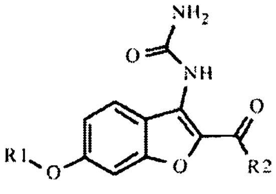
137



138



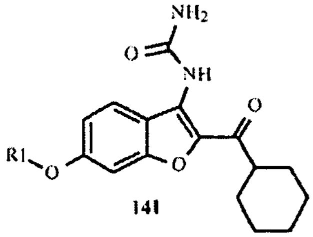
139



140

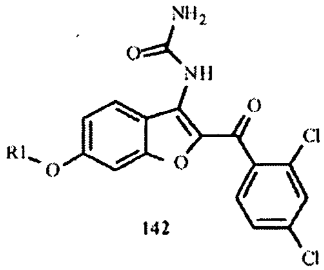
R1 = H, CH_2-Ph, CH_3, SO_2CH_3, CH_2-C(CH_2)-CH_2OH, CH_2CHCH_2, $CH_2CH(OH)CH_2OH$

R2 = c-C_3H_5, c-C_4H_7, c-C_5H_9, c-C_7H_{11}



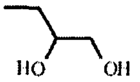
141

the rolipram 3,4-dialkoxy subunit. More recently, Celltech-Chiroscience reported the introduction of new 7-methoxybenzofuran-4-carboxamides with different substituents at the 2-position of the benzofuran nucleus (**137**) [334-335]. Among the compounds **137**, the ketone derivative was found to be the most potent PDE4 inhibitor with an IC_{50} value at 0.0016 μM in comparison to the rolipram IC_{50} at 3.5 μM. This compound provided an important selectivity for the catalytic site versus the rolipram binding site (LARBS/HARBS = 0.037). Then, the replacement of the 3,5-dichloropyrid-4-yl with various 6-membered aromatic rings was evaluated. Only, the 3-chloropyrid-4-yl derivative was in the same range of activity than the corresponding the 3,5-dichloropyrid-4-yl analog but with a lower LARBS/HARBS ratio. Administered at 0.5, 1, and 10 mg/kg *p.o.*, the 2-acetyl-7-methoxy-benzofuran-4-carboxylic acid (3,5-dichloro-pyridin-4-yl)-amide showed good activity across a variety of inflammation-induced

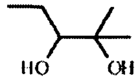


142

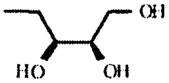
R1 =



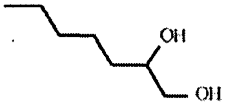
PDE4 IC_{50} = 0.006 μM
O_2-IC_{50} = 0.008 μM



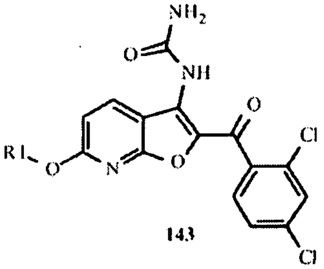
PDE4 IC_{50} = 0.05 μM
O_2-IC_{50} = 0.07 μM



PDE4 IC_{50} = 0.015 μM
O_2-IC_{50} = 0.01 μM

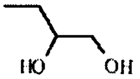


PDE4 IC_{50} = 0.042 μM
O_2-IC_{50} = 0.047 μM

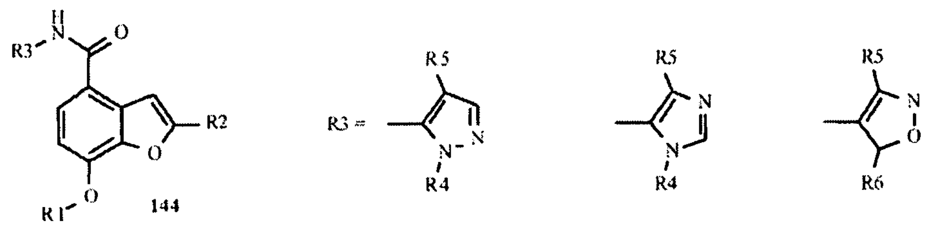


143

R1 =



PDE4 IC_{50} = 0.01 μM
O_2-IC_{50} = 0.008 μM



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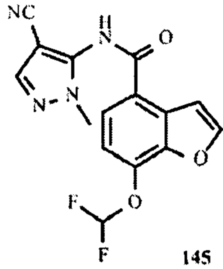
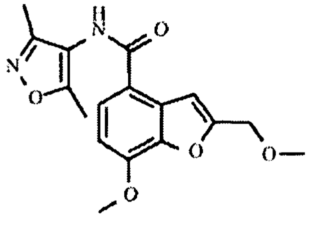


145



146

mediators in a guinea-pig skin eosinophilia model. Emesis and CNS effects were not observed with this compound in ferrets at the oral dose of 10 mg/kg.

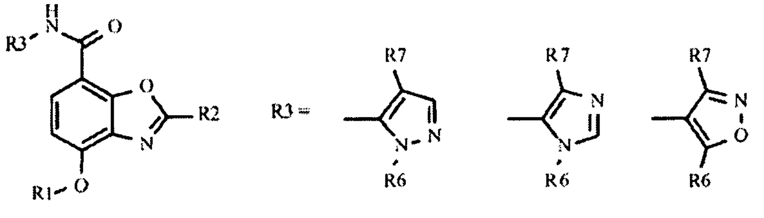
Very recently, Bayer developed new chemical series structurally related to the benzofuran nucleus. Novel benzofuranylaminoalcohols were reported as potent PDE4 inhibitors with HARBS IC_{50}s between 0.01 to 10 μM [336]. The specified candidate (**138**) showed a PDE4 IC_{50} value at 170 nM and inhibited the fMLP-induced superoxide production with an IC_{50} value at 80 nM.

In the same time, a series of new benzofuranylsulfonates was described [337]. One of the most potent compounds (**139**) provided an IC_{50} value at 1 nM in inhibiting PDE4 and in blocking fMLP-induced superoxide production.

Bayer also disclosed a novel series of cycloalkyl substituted 3-urea-benzofuranes (**140**) and of pyridofurane-derivatives [338]. The compounds were reported to inhibit PDE4 activity and fMLP-induced superoxide production with IC_{50} values in a range from 0.001 to 10 μM and from 0.07 to 10μM, respectively. The specified compound was *N*-(2-cyclohexylcarbonyl-6-methoxybenzofuran-3-yl)urea (**141**).

Finally, a new series of oligohydroxyl substituted benzofuran-3-yl (**142**) and pyridofuranylurea derivatives (**143**) was designed [339]. Several compounds were exemplified with their biological data.

New benzofuran carboxamides (**144**) such as **145** and **146** were reported to be TNF-α and PDE4 inhibitors by Darwin Discovery [340]. No biological data were reported.



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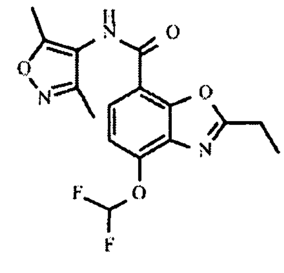

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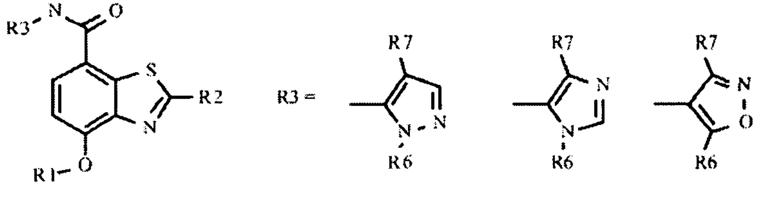

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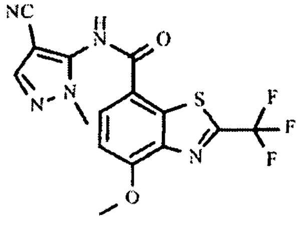

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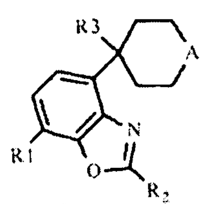

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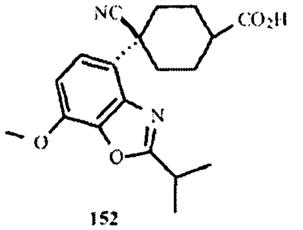
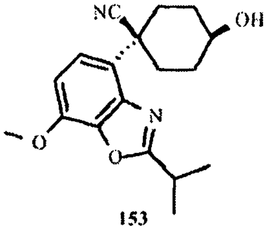


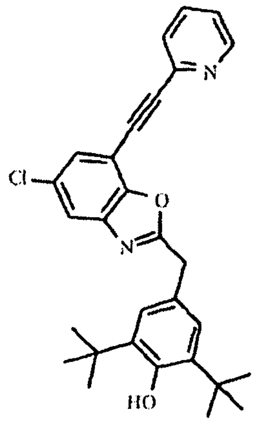
152
PDE4 $pIC_{50} = 7.69$



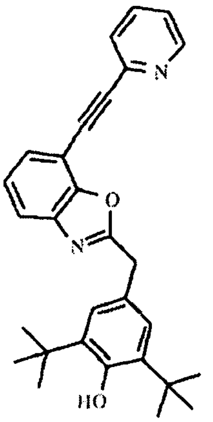
153
PDE4 $pIC_{50} = 7.77$

Darwin Discovery investigated novel analog series closely related to these benzofuran carboxamides with the corresponding benzoxazoles (**147**) such as **148** and benzothiazoles (**149**) such as **150** [341-342].

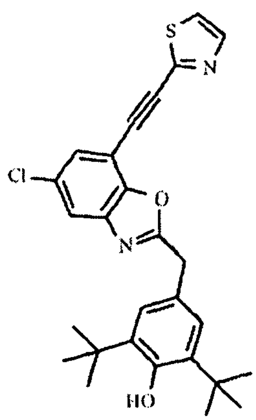
A new class of benzoxazoles (**151**) was disclosed in a patent from Byk Gulden [343]. Most of these compounds might be regarded as hybrid molecules of cilomilast and benzoxazoles. It was reported that the introduction of a hydroxyl (**153**) or carboxylic acid group (**152**) at the 1-position of the cyclo-hexane increased the solubility potency of such compounds. Moreover, the carboxylic acid derivative (**152**) was by far much more active than its corresponding methyl ester analog with a PDE4 $pIC_{50}s$ at 7.69 and 6.46, respectively.



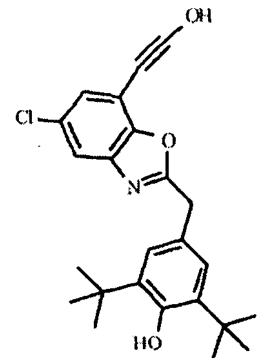
154
PDE4 $IC_{50} = 0.013$ μM



155
PDE4 $IC_{50} = 0.7$ μM



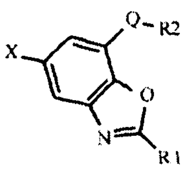
156
PDE4 $IC_{50} = 0.014$ μM



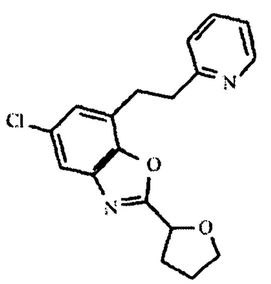
157
PDE4 $IC_{50} = 0.42$ μM

6,5-fused aromatic ring systems including several novel benzoxazole derivatives, acting as selective PDE4 inhibitors, were recently patented by Euroceltique [344]. The compounds, such as compounds **154-157** exhibited higher *in vitro* PDE4 activity and selectivity than rolipram (PDE4 IC_{50} = 3.7 μM) and were also more active to providing bronchodilating and anti-inflammatory effects.

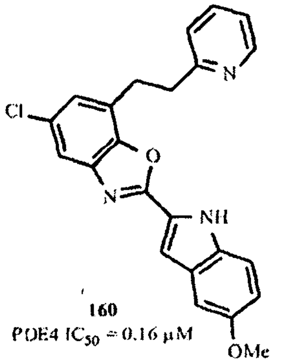
Euroceltique disclosed another new series of benzoxazoles (**158**) [345]. The most potent benzoxazoles were reported to be PDE4 inhibitors with $IC_{50}s$ between 0.16 and 4 μM (in comparison to 3.7 μM for the rolipram). The compounds **159** and **160** were among the most active candidates *in vitro*.



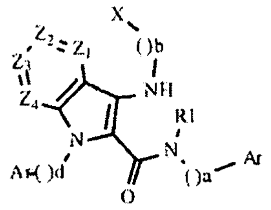
158



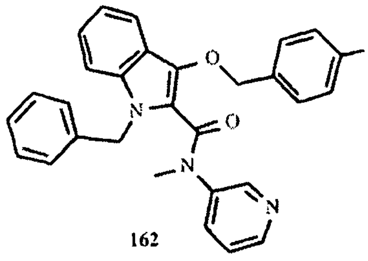
159
PDE4 $IC_{50} = 0.19$ μM



160
PDE4 $IC_{50} = 0.16$ μM



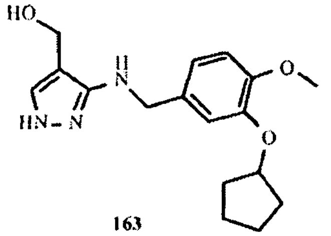
161



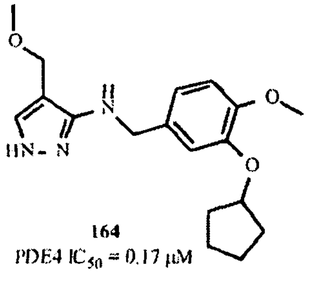
162

The indole-based scaffold was investigated by Merck Frost for the synthesis of new potent PDE4 inhibitors (**161**) such as compound **162** [346]. No biological data were reported.

Euroceltique also reported the synthesis of novel aryl pyrazoles. These derivatives provided similar or improved PDE4 inhibition in comparison to rolipram [347]. The preferred compounds were 3-(3-cyclopentyloxy-4-methoxy-benzylamino)-*1H*-4-hydroxymethyl pyrazole (**163**) and 3-(3-cyclopentyloxy-4-methoxy-benzylamino)-*1H*-4-methoxymethyl pyrazole (**164**) providing PDE4 IC_{50} values at 0.016 and 0.17 μM, respectively in comparison with the rolipram PDE4 IC_{50} at 4.5 μM.



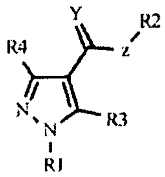
163
PDE4 IC_{50} = 0.016 μM



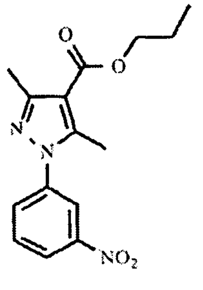
164
PDE4 IC_{50} = 0.17 μM

Novel pyrazoles derivatives (**165**), showing PDE4 and TNF-α inhibitory activities, useful for the treatment of inflammatory disorders were claimed by Icos [348]. The IC_{50} values of 33 compounds were reported between 0.053 and 180.67 μM. Nine compounds showed a LPS-stimulated TNF-α inhibition with IC_{50}s in a range from 0.52 to 6.2 μM. 1-cyclohexyl-3,5-dimethyl-1*H*-pyrazole-4-carboxylic acid ethyl ester (**167**) displayed IC_{50} value of 0.053 μM against human recombinant PDE4B and EC_{50} at 0.52 μM in an inhibition model of LPS-stimulated TNF-α release from human peripheral blood lymphocytes. 3,5-dimethyl-1-(3-nitrophenyl)-1*H*-pyrazole-4-carboxylic acid ethyl ester (**166**) provided a PDE4 inhibition with an IC_{50} value at 0.1 μM and an efficacy *in vivo* in an LPS mouse model with 70% and 100% inhibition respectively at 10 and 100 mg/kg by intraperitoneal injection. The compounds of the invention were also claimed to reduce or eliminate adverse CNS side effects and to produce a minimal or no emetic response.

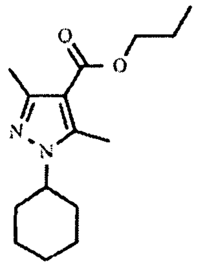
Icos recently claimed new series of pyrrolidine derivatives to be PDE4 inhibitors. New hydrazone and



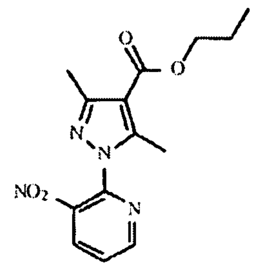
165



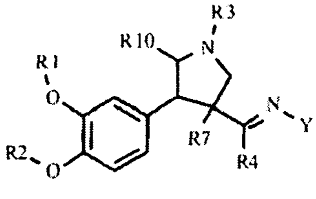
166
hrecPDE4 IC_{50} = 0.1 μM
% TNF-α inhibition: 70% at 10 mg/kg i.p. and 100% at 100 mg/kg i.p.



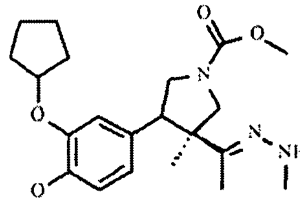
167
hrecPDE4 IC_{50} = 0.053 μM
PBL/TNF-α EC_{50} = 0.52 μM



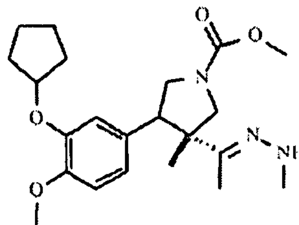
168
hrecPDE4 IC_{50} = 0.2 μM
PBL/TNF-α EC_{50} = 2 μM



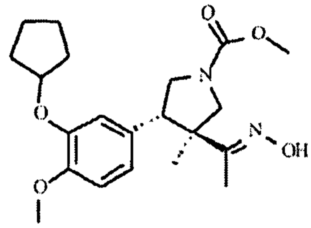
169



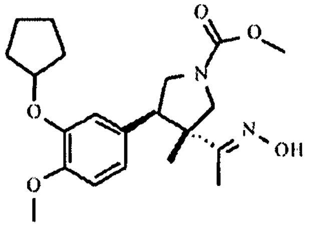
170 (4S)
hrecPDE4 IC_{50} = 115.9 nM
PBL/TNF-α EC_{50} = 76 nM



171 (4R)
hrecPDE4 IC_{50} = 2.2 nM
PBL/TNF-α EC_{50} = 2.2 nM



172 (3S, 4S)
hrecPDE4 IC_{50} = 49 nM
PBL/TNF-α EC_{50} = 281 nM



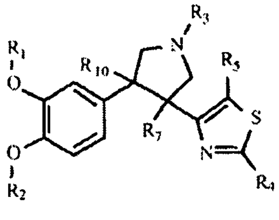
173 (3R, 4R)
hrecPDE4 IC_{50} = 2.2 nM
PBL/TNF-α EC_{50} = 23 nM

oxime analogs of pyrrolidine (**169**) were described in WO-0146136 [349]. The 4 compounds were evaluated using a combined mouse endotoxin-stimulated TNF-α release and locomotor activity assay. The percentages of activity (mobility), at 50 mg/kg *i.p.*, at 64% and 6% for **170** and **171**, respectively, were not related to the *in vitro* PDE4 data. However, accordingly to the *in vitro* PDE4 results, the ED_{50} values for **173** and **172**, respectively, were at 7 mg/kg and > 50 mg/kg.

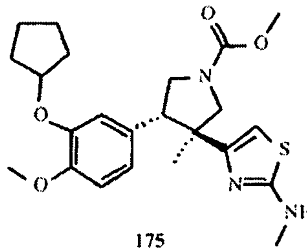
Novel 3-thiazol-4-ylpyrrolidines (**174**) such as **175** were reported in WO-0146184 [350]. Close analogs (**176**) such as **177** were also reported in WO-0147914 [351].

New pyrrolidines derivatives (**178**) such as **179** were claimed in WO-0147879 [352].

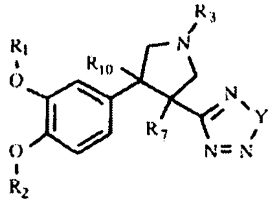
In WO-0147905 and in WO-0147915, Icos disclosed PDE4 pyrrolidines (**180** and **185**) structurally close to the previous series [353-354]. The compounds were claimed for their PDE4 and TNF-α inhibitory potency and for their ability to reduce CNS and emetic side effects. The compounds **181** and **182** provided similar PDE4 and LPS-stimulated TNF-α release inhibition. However, the 2 compounds showed very different behavioral profiles. Therefore, it seemed that the absolute configuration of such PDE4 inhibitors significantly contributed to discriminate



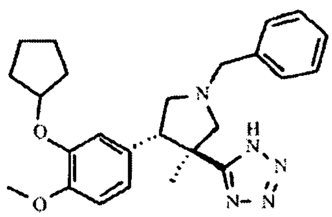
174



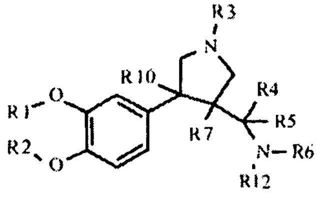
175
hrecPDE4 IC_{50} = 4.73 µM



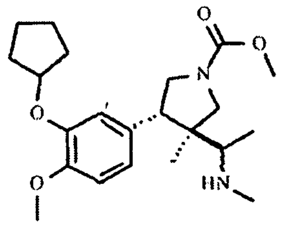
176



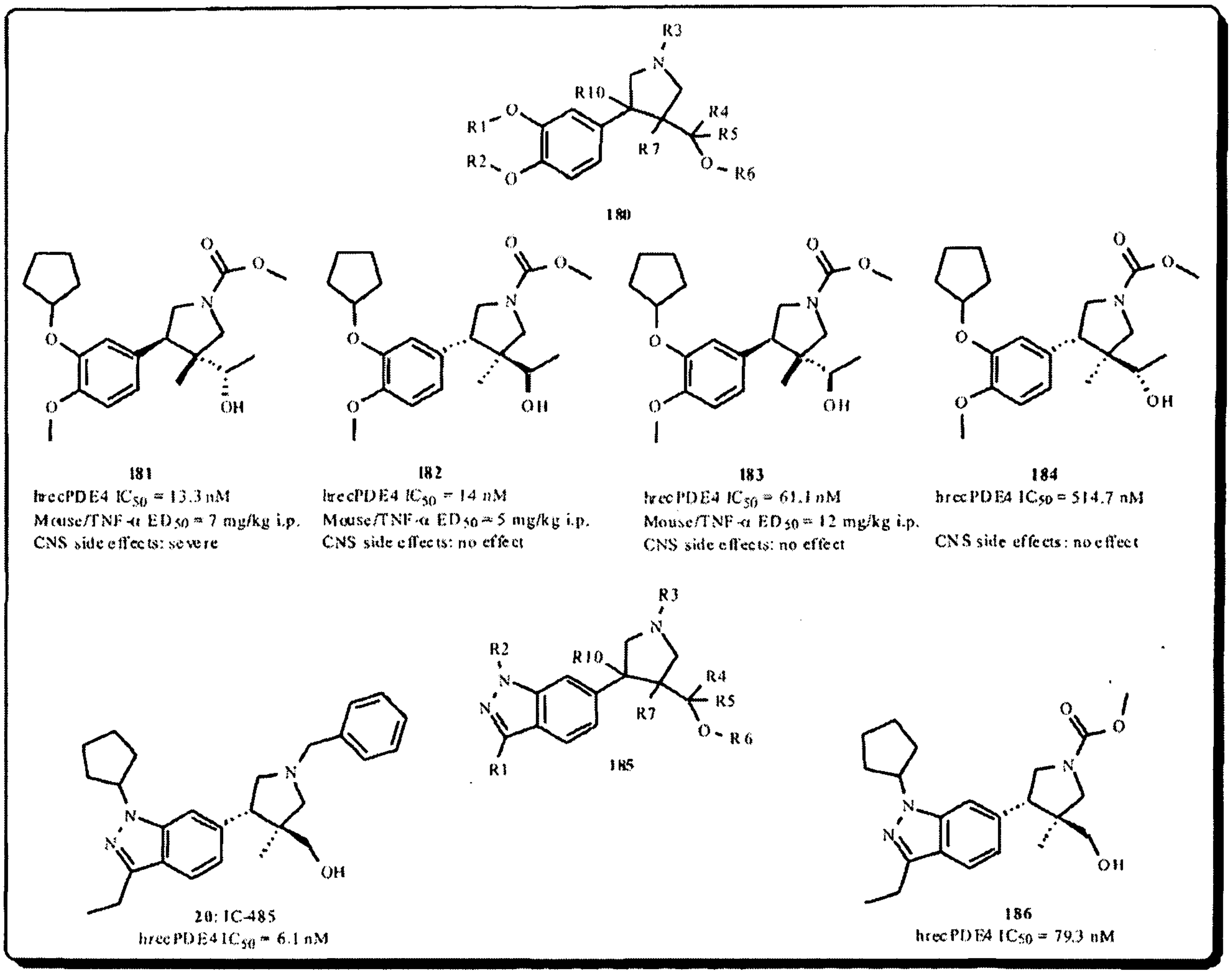
177
hrecPDE4 IC_{50} = 7.9 µM



178



179 = (3S, 4S) mixture of diastereoisomers
hrecPDE4 IC_{50} = 51.7 nM
PBL/TNF-α EC_{50} = 59.0 nM
% mobility at 50 mg/kg i.p. = 145%



180

181
hrecPDE4 IC_{50} = 13.3 nM
Mouse/TNF-α ED_{50} = 7 mg/kg i.p.
CNS side effects: severe

182
hrecPDE4 IC_{50} = 14 nM
Mouse/TNF-α ED_{50} ≈ 5 mg/kg i.p.
CNS side effects: no effect

183
hrecPDE4 IC_{50} = 61.1 nM
Mouse/TNF-α ED_{50} = 12 mg/kg i.p.
CNS side effects: no effect

184
hrecPDE4 IC_{50} = 514.7 nM
CNS side effects: no effect

185

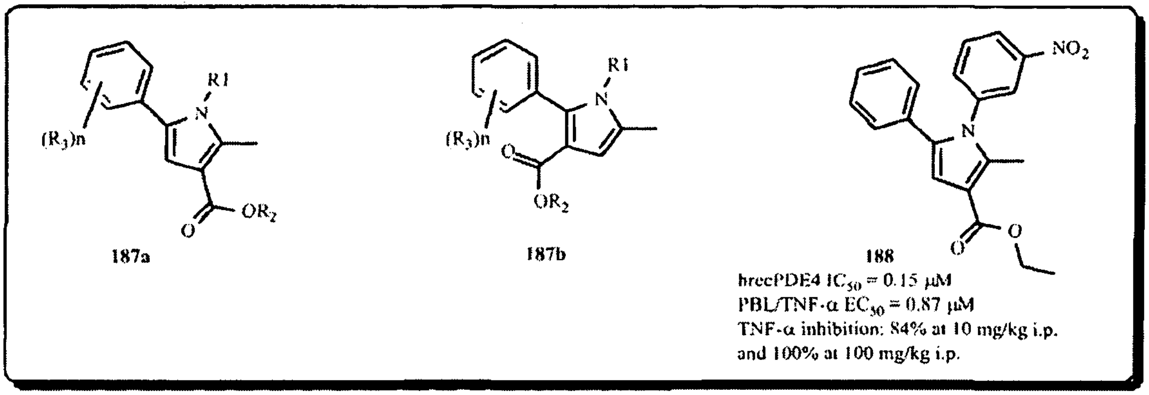
20: IC-485
hrecPDE4 IC_{50} = 6.1 nM

186
hrecPDE4 IC_{50} = 79.3 nM

efficacy and CNS side effect propensity. Both compounds **181** and **182**, after oral or intravenous injection (2.5 to 25 mg/kg and 1 to 5 mg/kg, respectively) produced a dose response of the emetic behavior. Compound **181** was by far much more emetic than compound **182**. Recently, IC-485 (**20**), was reported to begin a phase 1 trial for the oral treatment of inflammatory conditions [chapter on clinical candidates, 266].

Icos extended its PDE4 research through the investigation of novel pyrrole derivatives (**187a-b**) [355]. These compounds, such as **188**, were claimed to be PDE4 and TNF-α inhibitors. In addition, they were reported to reduce adverse CNS side effects and to induce a minimal or no emetic responses.

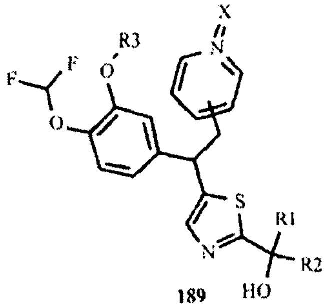
Merck Frost investigated a novel series of thiazole derivatives (**189**) [356]. The candidates were triaryl-substituted ethane analogs. The *in vitro* IC_{50} values with regards to the cAMP hydrolysis inhibition were in a range between 0.01 and 20 µM. *In vivo*, these compounds were active in reducing eosinophilia and accumulation of other



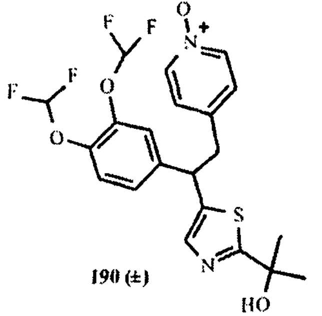
187a

187b

188
hrecPDE4 IC_{50} = 0.15 µM
PBL/TNF-α EC_{50} = 0.87 µM
TNF-α inhibition: 84% at 10 mg/kg i.p.
and 100% at 100 mg/kg i.p.



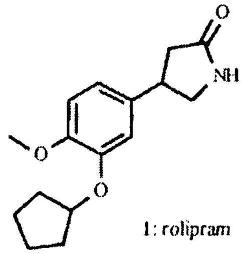
189



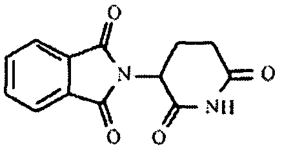
190 (±)

inflammatory leukocytes after oral or intraperitoneal dosages from 0.001 to 10 mg/kg. No specific data were presented.

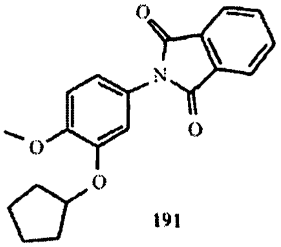
Isoindolindiones or Thalidomide Analogs



1: rolipram



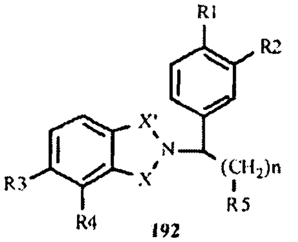
24 : thalidomide



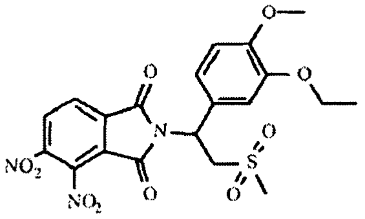
191

Thalidomide (**24**) was described as a specific inhibitor of a LPS-induced TNF-α production [357]. Therefore, thalidomide analogs were synthesized and demonstrated a good correlation between TNF-α inhibition and PDE4 inhibition. In 2000, Daewoong investigated the TNF-α inhibitory effect of a novel series of compounds designing through a combination between rolipram (**1**) and thalidomide (**24**) [358]. 2 - [3-(cyclopentyloxy)-4-methoxyphenyl]-1,3-isoindolin-dione (**191**) displayed a potent *in vitro* TNF-α inhibition in LPS-stimulated RAW264.7 cells with an IC_{50} at 0.68 μM in comparison to IC_{50}s at 0.1 μM and 194 μM for rolipram (**1**) and thalidomide (**24**), respectively.

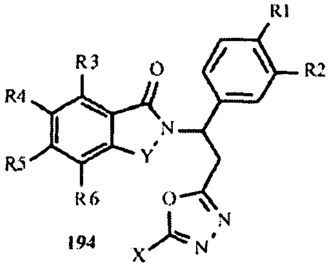
In the past, Celgene described modifications of thalidomide derivatives for enhancing TNF-α inhibitory activity. More recently, Celgene synthesized novel thalidomide analogs (**192, 194, and 196**) such as **193, 195** such **197**, respectively. The compounds **192** were found to display multiple potencies including TNF-α antagonism, matrix metalloproteinase inhibition and PDE4 inhibition [359]. The oxadiazoles **194** and substituted acylhydroxamic acids **196** were reported to be PDE4 and TNF-α inhibitors [360-361]. No biological data were reported.



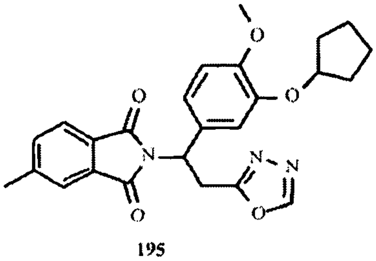
192



193



194



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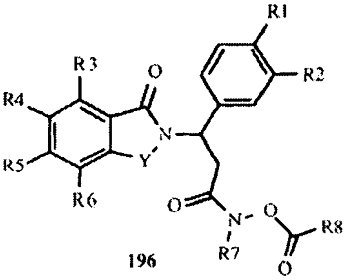
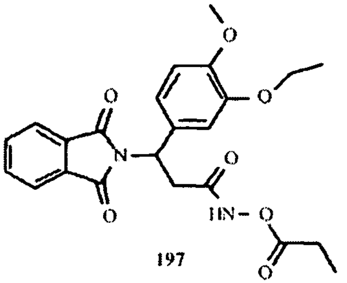
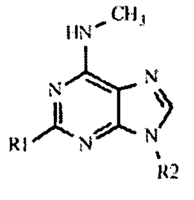
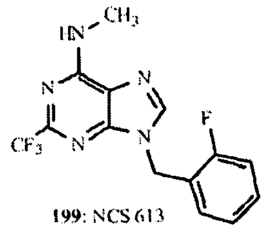


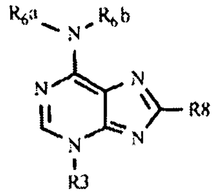
196

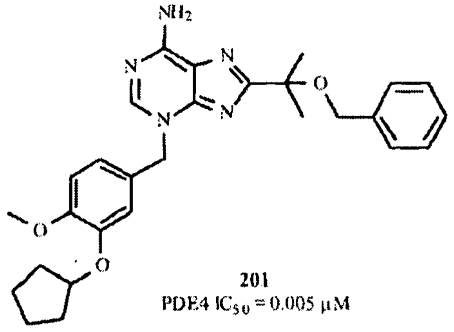


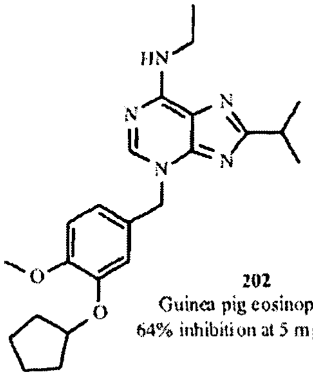
197

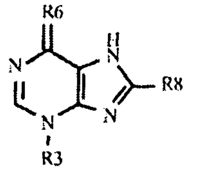

198

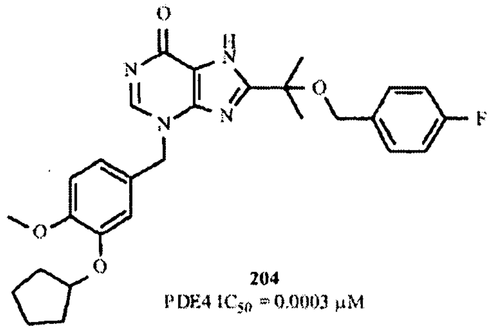

199: NCS 613

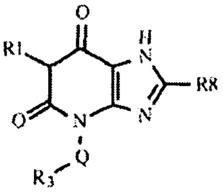

200


201
PDE4 IC_{50} = 0.005 μM


202
Guinea pig eosinophilia:
64% inhibition at 5 mg/kg i.p.


203


204
PDE4 IC_{50} = 0.0003 μM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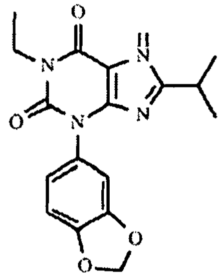
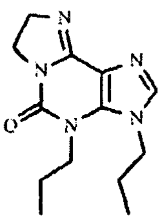

206

Benzyladenines, Purines and Condensed-Purines

Adenine derivatives substituted at 9-position (**198**) were found to provide a highly selective PDE4 inhibition [362]. These compounds were compared to RP-73401. The most promising compound, NCS-613 (**199**) was further evaluated *in vivo*. NCS-613 (**199**) dose dependently (1, 10, and 30 mg/kg *p.o.*) inhibited the LPS-induced neutrophil recruitment in mice BAL fluid. After intravenous administration of NCS-613 from 0.3 to 30 mg/kg, no gastric acid secretions in rats were observed suggesting that such a compound may produce an improved therapeutic index versus the first-generation of compound (RP-73401).

Euroceltique reported new series of purine derivatives (**200**, **203**, and **205**) [363-365]. Such compounds having a PDE4 activity could be useful for the treatment of several diseases including asthma, allergies and inflammation.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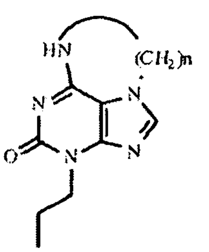
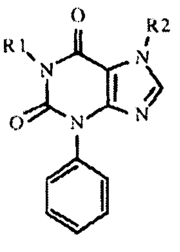
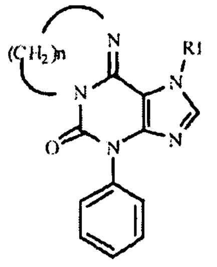

208
n = 3
n = 4


209
R1 = nBu, R2 = CH_2COCH_3
R1 = H, R2 = H
R1 = H, R2 = nPr
R1 = nBu, R2 = H


210
n = 2, R1 = nPr
n = 2, R1 = H
n = 3, R1 = nPr
n = 3, R1 = H

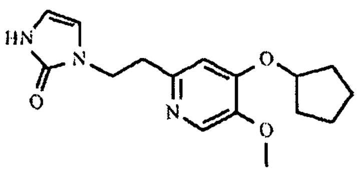
In WO-0059449, 8-(1-Benzyloxy-1-methyl-ethyl)-3-(3-cyclopentyloxy-4-methoxy-benzyl)-3*H*-purin-6-ylamine (**201**) was much more active than the standard theophylline (PDE4 IC_{50} = 1 μM). [3-(3-Cyclopentyloxy-4-methoxy-benzyl)-8-isopropyl-3*H*-purin-6-yl]-ethyl-amine (**202**) was evaluated *in vivo* showing 64% inhibition of eosinophils in guinea pig BAL at the *i.p.* dose of 5 mg/kg. In WO-0111967, a series of hypoxanthine derivatives was investigated. It was stated that such compounds could be potent PDE4 inhibitors, e.g. compound **204** providing a PDE4 IC_{50} value at 0.0003 μM.

In the past, 3,4-dipropyl-4,5,7,8-tetrahydro-3*H*-imidazo [1,2-*i*]purin-5-one (**207**) was described with a selective PDE4 activity. Then, novel condensed-purines (**208**) were designed with the aim of avoiding the xanthime-related adverse effects [366]. The two derivatives with n = 3 and n = 4 were synthesized and found not active on PDE4. Recently, the synthesis of 3-phenylxanthines (**209**) and 4-phenyl[*i*]condensed-purines (**210**) was performed [367]. In both series, compounds showing a PDE4 activity were identified. In the 3-phenylxanthine series (**209**), the derivative with R1 = nBu and R2 = H was the most active on PDE4 with an IC_{50} value at 9.4 μM. In the 4-phenyl[*i*]condensed-purine series (**210**), only the derivatives with R1 = nPr provided a PDE4 activity with IC_{50}s at 15 and 5.7 μM, respectively for n = 2 and n = 3.

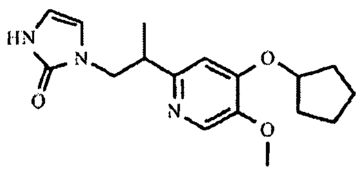
Other Series: Pyridines, Nicotinamides, Benzamides, Arylsulfone Hydroxamates

Janssen described a new series of dialkoxy-pyrydylalkyimidazolidines as selective PDE4 inhibitors [368]. It was reported that a pyridyl group within the catechol pharmacophore might lead to PDE4 active compounds (**211-214**).

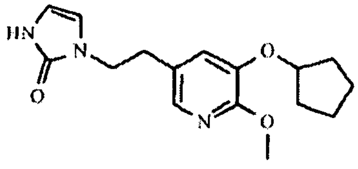
Dainippon disclosed a new class of 2,3-disubstituted pyridines with potent PDE4 activity and good bronchodilating propensity [369]. Therefore, the compounds such as **215** could be used to treat or prevent allergic and inflammatory status. 4-{3-[2-(3-Chloro-phenoxy)-pyridi-3-yloxy]-propyl}-pyridin-3-ol (**215**) inhibited guinea pig PDE4 with an IC_{50} at 15.7 nM. *In vivo*, the compound dose-dependently (3-30 mg/kg) inhibited the antigen-induced bronchoconstriction after oral administration.



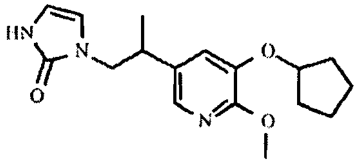
211
PDE4 : 56% inhibition at 1μM



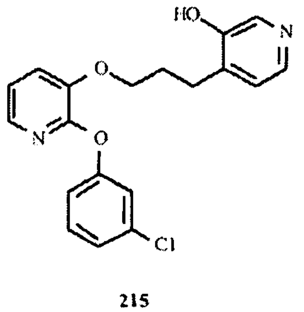
212
PDE4 : 60% inhibition at 100 nM



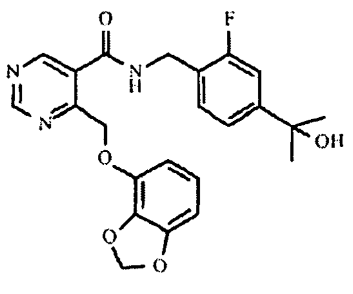
213
PDE4 : 75% inhibition at 100 nM



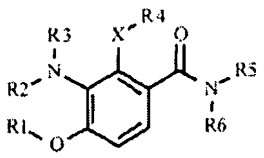
214
PDE4 : 59% inhibition at 100 nM



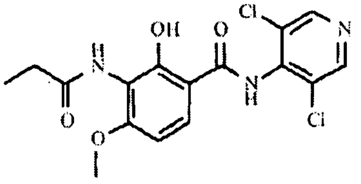
215



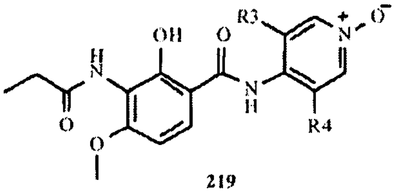
216



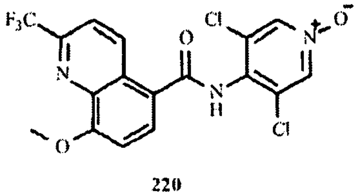
217



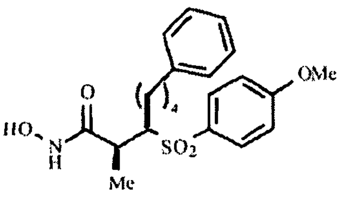
218



219

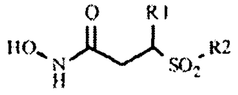


220

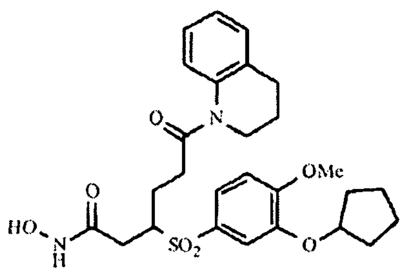


221

PDE4 IC_{50} = 0.03 μM
MMP-1 Ki = 2 μM
MMP-2 Ki = 0.01 μM
MMP-3 Ki = 0.5 μM



222



223

PDE4 IC_{50} = 0.016 μM
MMP-1 IC_{50} > 10 μM
MMP-2 IC_{50} > 10 μM
MMP-3 IC_{50} > 10 μM

New nicotinamide derivatives such as the pyrimidine carboxamide **216** were disclosed as PDE4 inhibitors by Pfizer [370-371]. *In vitro*, the bronchospasmolytic activity was found with IC_{50}s in a range between 0.001 and 1 μM. *In vivo*, the compounds were stated to exhibit in guinea pigs a bronchodilator activity at doses from 0.001 to 0.1 mg/kg *i.v.* or from 0.1 to 5 mg/kg i.d.: no specific biological data were provided.

In the WO patent applications, WO-0048998 and WO-0026208, novel aminobenzamide and *N*-oxide derivatives (**217** and **219** respectively) were disclosed by Darwin Discovery [372-373]. The compounds **217** and **219** were reported to be selective PDE4 inhibitors and blockers for TNF-α production and eosinophil accumulation. The compounds **219** such as **220** showed improvements with regards to the solubility, the metabolic stability and the pharmacokinetic profile. No biological data were mentioned.

The first compounds simultaneously inhibiting both PDE4 and MMPs 1, 2 and 3 were reported in 1999 by Rhône-Poulenc Rohrer and were represented by the β-(arylsulfonyl)hydroxamic acid (**221**) [374]. A solid-phase synthesis of an arylsulfone hydroxamate lead optimization library (**222**) was further described and the biological activity data directly obtained from the library, without any further purification, showed the potential of this type of the accelerating optimization process [375]. The introduction of a 3,4-dialkoxy group on R2 position provided selective PDE4 inhibitor with a nanomolar range of activity. In order to increase the PDE4 activity, the replacement of the 3,4-dimethoxy-phenyl group by the 3-*O*-cyclopentyl-4-*O*-methtyl-benzene moiety was appropriate in some cases. The modifications of the R1 group showed that steric and polar substituents led to acceptable PDE4 activity.

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Therapeutic benefit of PDE4 inhibitors in inflammatory diseases

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Intracellular levels of cyclic nucleotides are closely regulated by distinct families of PDEs, which are responsible for the breakdown and degradation of cyclic nucleotides within cells. Type 4 PDEs have the potency to modulate the release of inflammatory mediators through cAMP-dependent and -independent mechanisms. Selective targeting of PDE4 is currently being investigated as a novel therapeutic approach in the treatment of inflammation-associated respiratory diseases such as asthma and COPD. The development of several PDE4 inhibitors, including roflumilast and cilomilast, reflects the success of this approach. In principle, therapeutic intervention of an inflammatory response by PDE4 inhibitors may be extended to other chronic inflammatory disease states such as psoriasis, rheumatoid arthritis and inflammatory bowel diseases (eg, Crohn's disease and ulcerative colitis). This review explores the feasibility of PDE4 inhibitors as a promising alternative for therapeutic intervention in systemic inflammation and inflammation-based disease.

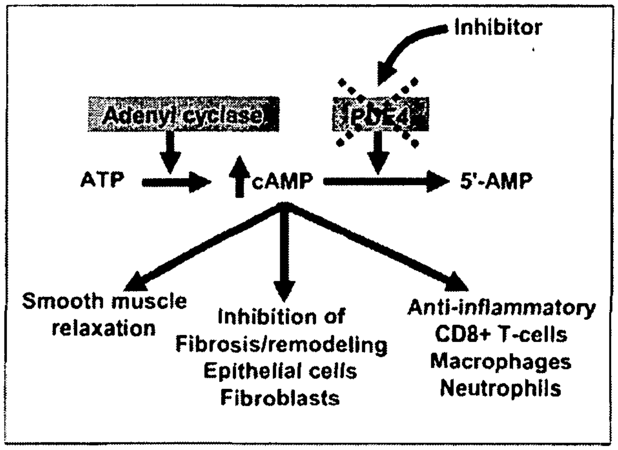
Keywords Arthritis, cAMP, inflammation, inflammatory bowel disease, inhibition, PDE4

Introduction

The second messenger cAMP, generated cytosolically by adenylate cyclases, is pivotal to a myriad of cellular functions. Intracellular alterations in cAMP are translated into cellular responses through cAMP-dependent protein kinase A (PKA). Signaling responses mediated by cAMP are compartmentalized and temporal cAMP gradients allow for the activation of spatially distinct pools of PKA-mediated regulation [1]. Various PKA isoforms are anchored at specific intracellular sites by A-kinase anchoring proteins that allow for discrete PKA populations to respond to cAMP gradients and modify localized target proteins [2,3]. The characteristics of cAMP gradients are dependent upon the activity of adenylate cyclases, which generate cAMP and PDEs that tailor cAMP degradation. The PDE superfamily of enzymes comprises at least 11 members (Table 1) capable of mediating hydrolysis of cyclic monophosphate nucleotides such as cAMP and cGMP [4,5].

PDEs are cyclic nucleotide hydrolyzing enzymes that are spatially distributed in various tissues. While there is a wide distribution of these enzymes inflammatory cells critical to the pathogenesis of inflammatory diseases, including asthma and COPD, preferentially express PDE4. This finding led to the search for subtype-selective inhibitors of PDEs [6-8]. The clinical efficacy of non-selective PDE4 inhibitors (eg, theophylline) provided further evidence for investigating the pharmacological control of intracellular levels of cAMP in proinflammatory and immunocompetent cells [9-11], and for PDE4 as an effective therapeutic target in a variety of inflammation-based diseases. The mechanism of action of PDE4 inhibitors is summarized in Figure 1.

Figure 1. Mechanism of action of PDE4 inhibitors.



PDE4 subtypes represent the major class of PDEs expressed in a variety of cells, particularly macrophages, eosinophils and neutrophils, which are central to the initiation and progression of an inflammatory immune response. The therapeutic approach is based on the chronic inflammatory nature of certain diseases and the observation that increasing cyclic nucleotides via PDE inhibition leads to the alleviation of various proinflammatory parameters [12,13,14••]. Positive results from pharmacological studies and clinical trials evaluating the efficacy of selective PDE4 inhibitors in respiratory diseases validate this hypothesis [15-17].

Inflammation is the basic response to infection and/or external and internal injury. Both inflammatory and autoimmune diseases are often associated with deregulated expression and biosynthesis of inflammatory cytokines, which influence a plethora of cellular functions. Down-modulation of an inflammatory signal concomitant with amplification of a counter anti-inflammatory signal would provide a rational approach to the treatment of inflammatory diseases. Insights into the molecular mechanisms of the regulatory role of cytokines in the amplification of an inflammatory cascade may also provide a new approach for pharmacological intervention.

PDE4 inhibition and inflammation

The chemotherapeutic potential of PDE4 inhibition is centered on the immunomodulatory properties of cyclic nucleotides. Nonspecific inhibition of PDEs by pentoxifylline results in a pharmacologically efficient modulation of immune functions and has clinical implications in regulating proinflammatory cytokines [18], and the treatment of bronchopulmonary dysplasia [19] and

Table 1. Classification of the PDE family.

PDE family	Substrate	Specific inhibitors	Property
PDE1	cAMP, cGMP	Nimodipine	Ca-calmodulin activated
PDE2	cAMP, cGMP	EHNA	cGMP activated
PDE3	cAMP, cGMP	Cilostamide, milrinone	cGMP inhibited
PDE4	cAMP	Rolipram, Ro-20-1724, Roflumilast (ALTANA Pharma AG/ Tanabe Seiyaku Co Ltd)	cGMP insensitive
PDE5	cGMP	Zaprinast, DMPPO, E-4021, sildenafil	PKA/PKG phosphorylated
PDE6	cGMP	Zaprinast, DMPPO, E-4021, sildenafil	Transducin activated
PDE7	cAMP	BRL-50481 (GlaxoSmithKline plc)	Rolipram insensitive
PDE8	cAMP	Unknown	Rolipram insensitive IBMX insensitive
PDE9	cGMP	BAY-736691	IBMX insensitive
PDE10	cAMP, cGMP	Unknown	Unknown
PDE11	cAMP, cGMP	BAY-656207	Unknown

DMPPO 1,3-dimethyl-6-(2-propoxy-5-methanesulfonylamidophenyl)pyrazol [3,4*d*]-pyrimidin-4-(5*H*)-one, **EHNA** erythro-9-(2-hydroxy-3-nonyl)adenine, **IBMX** 3-isobutyl-1-methylxanthine, **PK** protein kinase.

sepsis [20]. cAMP elevation in immunocompetent cells suppresses or inhibits the release of inflammatory mediators and relevant cytokines, as well as the recruitment and activation of inflammatory cells. Cytokines such as IL-1β and TNFα upregulate cell adhesion molecules such as P-selectin, E-selectin, ICAM-1 and vascular cell adhesion molecule [21,22]. PDE4 inhibitors abrogate several T-lymphocyte functions, and intercept T-cell activation and downstream signaling events. Agonists operating via the adenylate cyclase pathway or cell permeable cAMP analogs, inhibit T-cell activation by interfering with phosphatidylinositol turnover, which increases intracellular Ca^{2+} mobilization and protein kinase C activation [23-26]. Effects of the T-cell antigen-receptor (TCR) complex and transcription factors such as nuclear factor of activated T-cells, NFκB and activator protein-1 on phosphorylation have also been reported [27]. Inhibitory effects of cAMP on T-cell functions have been related to PKA-triggered phosphorylation and subsequent activation of the small tyrosine kinase Csk that inhibits Lck, a pivotal tyrosine kinase mediating TCR signaling [28,29]. The localization of PDE4 within the lipid raft signal following anti-CD28 costimulation may also form the basis for the efficacy of PDE4 inhibitors in preventing multiple T-cell functions triggered by anti-CD3/anti-CD28 costimulation [30]. Thus, the mechanics of inhibition are pertinent to allergen-induced T-cell activation and therefore of consequence to allergic disease. PDE4 inhibitors suppress the production of proliferation-driven Th1 and Th2 cytokines such as IFNγ, IL-2, IL-4, IL-5 and IL-13 [31,32], as well as reducing cytokine levels *in vivo* [33,34]. The effects of PDE4 inhibition in diverse cell types are summarized in Table 2.

Contradictory evidence exists on the effects of PDE4 inhibition on cytokine production. In Th1-driven disease states, the ability of PDE4 inhibitors to reduce cytokine production is greater for the Th1 cytokines IL-2 and IFNγ than for IL-4 or IL-5, indicating a greater clinical benefit of Th1 cytokines in autoimmunity [35,36]. However, in allergic disease models the inhibition of Th2 cytokines such as IL-5 was greater than that for Th1 cytokines such as IL-2, GM-CSF and IFNγ, indicating a preferential Th2 inhibitory potential [37]. The basis for sensitivity to inhibition of Th1 versus Th2 cytokines thus seems dependent on the disease model. Whether PDE4 inhibitors have increased therapeutic potential in typical Th1- (eg, rheumatoid arthritis [RA] and inflammatory bowel disease [IBD]) or Th2- (eg, asthma and atopic dermatitis) mediated diseases is still unclear.

PDE4 inhibitors in asthma and COPD

Over the last 15 years there has been much excitement in the suggestion that targeting PDE4 with small-molecule inhibitors could lead to the development of novel, steroid-sparing compounds with utility in a multitude of diseases associated with chronic inflammation, particularly asthma and COPD. Several candidate PDE4 inhibitors have been investigated, but only cilomilast (GlaxoSmithkline plc) and roflumilast (ALTANA Pharma AG/Tanabe Seiyaku Co Ltd) have proceeded to pre-registration and phase III clinical trials, respectively [38,39].

Table 2. Effects of PDE4 inhibitors on immune cells.

Cell type	Broad function	Effects of PDE4 inhibitors
Mast cells	Inflammation, mediator release	Recruitment/activation, degranulation
Eosinophils	Chemotaxis, mediator release	Superoxide generation, chemotaxis, degranulation, leukotriene C_4 synthesis
Macrophages	Mediator release	Leukotriene B_4 synthesis, TNFα release
Neutrophils	Chemotaxis, mediator release	Superoxide generation, infiltration
T-lymphocytes	Proliferation, cytokine release and polarization	Proliferation/cytokine inhibition
B-lymphocytes	Differentiation, Ig secretion	IgE production
Airway smooth muscle cells	Structural remodeling	Bronchoconstriction
Epithelial cells	Structural remodeling	Bronchoconstriction

Despite encouraging data from phase III trials of these compounds in COPD, their approval has been hampered by their low therapeutic ratio. Currently, GlaxoSmithKline lists cilomilast as 'approvable' [40] and ALTANA Pharma/ Tanabe Seiyaku are still investigating roflumilast in Japan [41]. There are a number of excellent reviews on the potential of PDE4 inhibitors for the treatment of asthma and COPD [42••,43••,44••,45••,46••,47], and thus they will not be discussed further in this review.

PDE4 inhibition in arthritis

Arthritis is the leading cause of physical disability worldwide and comprises over 100 different disease conditions, including RA, osteoarthritis, psoriatic arthritis, fibromyalgia and gout. RA is a chronic systemic autoimmune inflammatory disease characterized by nonspecific inflammation of the peripheral joints, potentially resulting in progressive destruction of articular and periarticular structures. Lymphocyte infiltration to the synovial tissue, as well as that of macrophages and their cytokines (eg, TNFα and GM-CSF), and increased expression of adhesion molecules, contribute to inflammatory cell emigration and retention in the synovial tissue, and disease pathogenesis [48,49].

Data from knockout mice demonstrated that TNFα and IL-1β are prominent in the pathogenesis of RA [50,51]. Genetically altered mice overexpressing TNFα spontaneously develop RA-like lesions in the joints with progressive inflammation and bone destruction [52,53]. However, disease development is abrogated by passive immunization with an anti-IL-1β receptor antibody suggesting an indirect role for TNFα [54]. Genetically susceptible DBA/1J mice show increased TNFα expression in the synovial lining of the inflamed joints following immunization with type II collagen, concurrent with the onset of symptoms [55]. It is unclear whether TNFα directly mediates this process and/or is a secondary effect linked to the expression of TNF-induced proinflammatory cytokines such as IL-1β and other chemokines [56,57]. Disease development may therefore result from the combined effect of dysregulated expression of several proinflammatory cytokines, such as TNFα and IL-1β, along with insufficient production of anti-inflammatory cytokines, such as IL-10.

The cAMP pathway represents a useful therapeutic target in RA due to its opposing effects on both TNFα and IL-10. Elevation of intracellular levels of cAMP in leukocytes by PDE4 inhibitors is accompanied by a significant inhibition of TNFα production at a transcriptional and translational level. Forskolin, an adenylate cyclase activator, also inhibits TNFα production. cAMP response element-binding (CREB) proteins are involved in synovial cell activity in patients with RA and inhibition of CREB activity corrects aberrant synovial cell functions in these patients [58]. Rolipram, a PDE inhibitor, ameliorates collagen-induced arthritis in murine experimental models [59]. Piclamilast and roflumilast, both selective PDE4 inhibitors, partially attenuate the IL-1β-induced nitric oxide production that contributes to cartilage degradation in osteoarthritis, suggesting that PDE4 inhibitors may also have chondroprotective effects [60]. While roflumilast is still undergoing clinical studies, the development of rolipram and piclamilast was discontinued in 2000 and 1996, respectively [61-63].

PDE4 activity is associated with a wide variety of inflammatory diseases and thus the regulation of proinflammatory cytokine production through PDE4 activity is being investigated for therapeutic utility in patients with RA. However, it must be noted that animal models of arthritis are induced and do not occur naturally; therefore, while they may demonstrate 'arthritis-like symptoms', the series of events are not truly reflective of human pathogenesis. For example, the collagen-induced rodent model for arthritis is based on the development of antibodies against collagen, a feature not observed in most clinical cases. This presents a serious limitation in understanding the disease-system dynamics and also the assessment of drugs and devising strategies for chemotherapeutic intervention. As a result, while preclinical data often support the clinical application of PDE4 inhibitors, the translation of this efficacy to success in trials can prove difficult.

Pathogenesis in IBD

Chronic IBDs are debilitating disorders of unknown etiology whose incidence is on the rise worldwide. Two main categories of IBD are ulcerative colitis and Crohn's disease [64,65]. Ulcerative colitis is characterized by recurring episodes of inflammation restricted to the mucosal layer of the rectum and may extend proximally. Crohn's disease is characterized by transmural inflammation of any part of the bowel, from the mouth to the anus. The prevalence of ulcerative colitis and Crohn's disease ranges from 10 to 70 per 100,000 individuals.

A variety of cytokines, such as TNFα, IL-1β, IL-6 and IL-8, contribute to immune stimulation, and cause intestinal tissue damage and T-helper (Th) cell differentiation. Th1 cells are associated principally with Crohn's disease, whereas Th2 cells are associated principally with ulcerative colitis. Existing therapies predominantly include aminosalicylates and steroids for both diseases; however, they are not useful for maintaining remission [66]. Current IBD therapy therefore utilizes nonspecific anti-inflammatory and immunosuppressive agents that are only partially effective and have serious side effects. Furthermore, a significant proportion of patients are either steroid resistant (~15 to 20%) or steroid dependent (~25 to 30%). Anti-TNFα has been explored as a therapy for IBDs, and infliximab (Remicade) became the first biological agent to be approved by the FDA for the treatment of IBD, and has since demonstrated efficacy for the acute management of inflammatory and fistulous Crohn's disease [67]. A PEGylated form of anti-TNFα antibody, CDP-870 (UCB SA, formerly Celltech), has demonstrated efficacy and is currently pre-registered in the US and Europe for Crohn's disease [68,69]. These developments have provided a rationale for the development of other biological agents that target the inflammatory cytokine pathway of the immune response.

The success of infliximab against Crohn's disease demonstrates TNFα as a functional therapeutic target. PDE4 inhibitors are potent suppressors of proinflammatory cytokines, particularly TNFα, suggesting that they could also be effective in IBD. PDE4 inhibitors stimulate the release of anti-inflammatory mediators such as IL-10 and PGE_2, which are major regulators of inflammation in the gut [70]. For example, cilomilast inhibits fibroblast-mediated collagen-gel degradation induced by TNFα and neutrophil elastase by suppressing the release of the matrix metalloproteases (MMPs) 1 and 9 [71]. Given that MMPs are associated with tissue destruction, this pathway may be an additional mode through which PDE4 inhibitors contribute to IBD therapies. PDE4 inhibitors also prevent and reverse gastrointestinal inflammation in several animal models. Their clinical benefit in respiratory disease is partly attributable to its modulation of airway remodeling. Tissue remodeling is an important feature of IBD and when extended to the gut epithelium could also translate to a therapeutic benefit in inflammation-based disorders of the gastrointestinal tract. Furthermore, the ability of PDE4 inhibitors to reduce the proinflammatory properties of leukocytes and maintain mucosal blood flow provides a spectrum of biological activities not observed with current medications. Tetomilast (Otsuka Pharmaceutical Co Ltd) is a small-molecule inhibitor of PDE4 that has shown promise in the treatment of ulcerative colitis, and is currently undergoing phase III clinical trials for this indication [72]. CC-1088, a thalidomide analog and PDE4 inhibitor, had completed a phase I/II double-blind, placebo-controlled trial in moderate-to-severe Crohn's disease which showed the compound to be safe and well tolerated [73]. However, this compound is no longer being developed by Celgene Corp, and the company is now focusing on another compound in the original series, CC-10004, for the treatment of inflammatory diseases (particularly psoriasis) [74]. PDE4 inhibition thus appears to be a viable therapeutic target for gut inflammation [70]. In addition, compounds that are either not absorbed into portal circulation or that undergo extensive metabolism (such as that indicated for budesonide) may have an improved safety profile over current therapies.

Inflammatory disorders of the skin

Psoriasis is a chronic inflammatory skin disorder caused by inflammatory cell infiltration into the dermis and epidermis, and is accompanied with keratinocyte hyperproliferation [75]. It is a complex disease and a combination of genetic and environmental factors are likely to be causative. Cytokines such as TNFα, IFNγ and chemokines orchestrate the development of psoriasis. TNFα is one of the primary cytokines that facilitates T-cell:antigen-presenting cell interaction by enhancing adhesion molecules such as ICAM-1 and thereby initiating the cascade of immune activation [76]. As PDE4 is also expressed in keratinocytes, these cells may be additional pharmacological targets of PDE4 inhibitors for the control of inflammatory skin disorders [77-80]. In fact, GlaxoSmithKline (under license from elbion AG) is developing a topical formulation of AWD-12-281 for the treatment of atopic dermatitis [81].

In addition to the indications mentioned above, PDE4 inhibitors have also been investigated for the treatment of age-related memory impairment and Alzheimer's disease [82], as well as skeletal muscle atrophy [83], hypertension [84] and autoimmune diseases [85]. However, their therapeutic benefits in these indications have yet to be demonstrated in the clinical setting.

Strategies to avoid the side effects of oral PDE4 inhibitors

The current PDE4 inhibitors in advanced stages of clinical development, cilomilast and roflumilast, have demonstrated encouraging efficacy in patients with COPD and have a superior safety/tolerability profile compared with nonselective PDE inhibitors such as theophylline [11] and first generation PDE4 inhibitors such as rolipram [14••]. The occurrence of side effects such as nausea, vomiting and gastric acid secretion has led to the search for new inhibitors with better efficacy profiles. Table 3 summarizes the current status of PDE4 inhibitors in various stages of development.

Table 3. Selected PDE4 inhibitors in development.

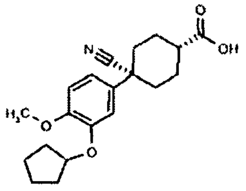
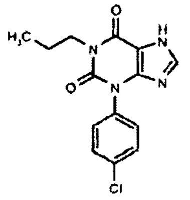
Compound	**Development status**	**Indication**
Cilomilast (GlaxoSmithKline plc)  	Pre-registration	COPD, asthma
Arofylline (Almirall Prodesfarma SA)  	Phase III trials	COPD, asthma, bronchitis

Table 3. Selected PDE4 inhibitors in development (*continued*).

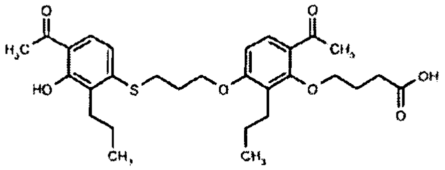
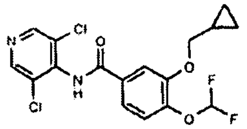
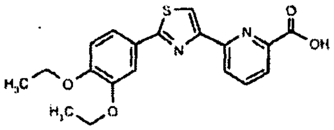
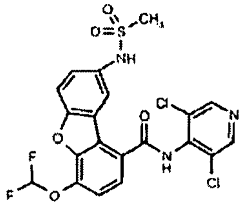
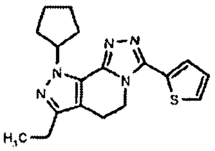
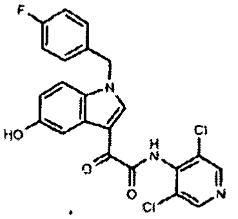
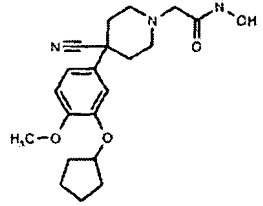
Compound	**Development status**	**Indication**
MN-001 (MediciNova Inc) 	Phase III trials	Asthma, cystitis
Roflumilast (ALTANA Pharma AG/Tanabe Seiyaku Co Ltd) 	Phase III trials	COPD, asthma
Tetomilast (Otsuka Pharmaceutical Co Ltd) 	Phase III trials	COPD, IBD, ulcerative colitis
256066 (GlaxoSmithKline plc)	Phase II trials	Allergic rhinitis, COPD, inflammation, asthma
C-3885 (Merck & Co Inc)	Phase II trials	Allergic rhinitis
CC-10004 (Celgene Corp)	Phase II trials	Inflammation, psoriasis, asthma
IPL-455903 (Helicon Therapeutics Inc)	Phase II trials	Cognition disorder
MEM-1414 (Memory Pharmaceuticals Corp)	Phase II trials	Alzheimer's disease
Oglemilast (Glenmark Pharmaceuticals Ltd/Forest Laboratories Inc/Teijin Ltd) 	Phase II trials	COPD, rheumatoid arthritis, asthma
Tofimilast (Pfizer Inc) 	Phase I/II trials	Allergy, respiratory disease
AWD-12-281 (GlaxoSmithKline plc) 	Phase I trials	Atopic dermatitis
EHT-0202 (ExonHit Therapeutics SA)	Phase I trials	Alzheimers disease, neurodegenerative disease
LAS-37779 (Almirall Prodesfarma SA)	Phase I trials	Psoriasis
ND-125 (Evotec AG, formerly Neuro3d SA)	Phase I trials	Major depressive disorder
A series of selective cytokine inhibitory drugs, including CC-11050 (Celgene Corp)	Discovery	Congestive heart failure, inflammation, autoimmune disease, cancer, respiratory disease

Table 3. Selected PDE4 inhibitors in development (*continued*).

Compound	Development status	Indication
ONO-6126 (Santen Pharmaceutical Co Ltd) 	Discovery	Allergy, conjunctivitis
CC-7085 (Celgene Corp)	No development reported	Inflammation
KW-4490 (Kyowa Hakko Kogyo Co Ltd)	Discontinued	Asthma

The therapeutic promise of PDE4 inhibitors has been tempered due to the development of side effects, particularly nausea, emesis and headache, and potentially fatal conditions such as vasculitis and colitis [86]. All PDE4 inhibitors are emetic to a certain degree, suggesting that cAMP elevation in the emetic centers triggers an emetic response [87,88].

A correlation of low-affinity rolipram-binding site (LARBS) with inhibition of cAMP hydrolysis is related to the anti-inflammatory properties, while the high-affinity rolipram binding site (HARBS) correlates with the production of certain side effects [89]. Rolipram binds with high affinity to HARBS and low affinity to LARBS and consequently is highly emetic at therapeutic doses [90]. Second-generation PDE4 inhibitors such as cilomilast and roflumilast, however, have comparable affinities to HARBS and LARBS and hence an improved therapeutic window. Thus, drug development is faced with the challenge of alleviating the side-effect profile of PDE4 inhibitors by widening the window between efficacy and associated adversities. Identification of distinct pharmacological PDE4 forms may demonstrate improved therapeutic consequences, as it may be possible to synthesize potent inhibitors of PDE4 with low affinity for HARBS, which should, theoretically, be less emetic. However, this theory has been questioned by some investigators who feel it is too simplistic and should no longer be exploited in compound development [44••].

Another strategy involves the development of subtype-specific PDE4 inhibitors. PDE4 has four subtypes: A, B, C and D. PDE4B is the predominant PDE4 subtype in monocytes and neutrophils crucial in inflammation responses [91,92•]. PDE4D has a key role in balancing relaxation and contraction in airway smooth muscle for maintenance of airway tone [93•]. Both PDE4B and PDE4D also have complementary, non-redundant roles in the control of neutrophil function [94]. Experiments with mice deficient in the *PDE4B* or *PDE4D* gene demonstrate that emesis is linked to the selective inhibition of PDE4D [95•]. PDE4D3 has also been implicated in cardiac malfunction [96]. PDE4B$^{-/-}$ mice exhibit a profound attenuation of LPS-induced-TNFα release, which provides further rationale for exploring pharmacological inhibition of the enzyme for anti-inflammatory effects [92•]. Thus, the development of PDE4B isoform-specific inhibitors could alleviate the side effects triggered by nonspecific inhibition of PDE4D activity and thereby improve therapeutic advantage in inflammatory diseases.

Alternate possibilities to overcoming dose-limiting side effects associated with PDE4 inhibition are currently being explored. Localizing drug administration to the site of inflammation, as with the intratracheal/intranasal route of delivery for the treatment of respiratory disorders, is not only advantageous for molecules with low bioavailability, but may also overcome overt side effects. Novel dual-action based approaches combining the benefits of related therapeutic targets could also lower the effective doses of PDE4 inhibitors required to offer clinical benefit and thereby dispel unwanted systemic effects. PDE7, another cAMP-specific PDE enzyme, has also been implicated in inflammation and is present in many proinflammatory and immune cells [97]. In addition, PDE1C promotes smooth-muscle cell proliferation in arterial tissue [98]. Inhibitors which target both PDE4 and PDE7 have demonstrated a broad anti-inflammatory effect in both *in vitro* and animal models of inflammation [99,100]. Dual-specificity inhibitors which target PDE4 and PDE1C could retard smooth-muscle cell proliferation and affect airway remodeling which may provide an improved therapeutic index and better safety profile [101••].

Conclusions

Experimental evidence implicates the role of PDE4 in airway diseases, particularly the PDE4B subtype [92•]. The diversity in the regulation of cAMP and cGMP levels may be important for various other clinical conditions, which may thus benefit from pharmacological inhibition of PDE4. Preclinical data suggest that PDE4 inhibitors could be of clinical benefit to diverse diseases such as ulcerative colitis, arthritis and neuronal inflammation. The protective effects of tetomilast have been demonstrated in phase III clinical trials for ulcerative colitis as well as phase II clinical trials for COPD [82], and roflumilast [39] and rolipram [55] have shown efficacy in animal models of induced arthritis. Notably, the clinical efficacy of PDE4 inhibition is more concrete for Th2-mediated respiratory disorders and whether this will also benefit patients with Th1-mediated disorders remains to be established; however, preclinical evidence suggests that this might well be the case. PDE4 inhibition still requires further clinical investigation, especially when considering the possible side effect profiles associated with systemic exposure. Pharmaceutical companies are currently exploring the clinical benefit of a variety of PDE4 inhibitors and the results of these investigations should reveal the feasibility of these compounds as a 'pan-anti-inflammatory' therapeutics.

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